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SOLICITUD DE PATENTE DE INVENCIÓN

TITULO: PROTEINAS DE FUSION DE ALBUMINA

SOLICITANTE: HUMAN GENOME SCIENCE INC.

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A61K 38/02

APODERADO: J. IAN RAISBECK

BOGOTÁ 07 de septiembre de 2006

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OLARTERAISBECK

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SOLICITUD DE:

(1) [X] Patente de Invención. [ ] Patente de Modelo de Utilidad.

[X] Capítulo I. [ ] Capítulo II.

(2) **SOLICITANTE (71)**	**Nombre:** HUMAN GENOME SCIENCE INC. **Dirección:** 14200 Shady Grove Road, Rockville, Maryland 20850. E.U.A. **Nacionalidad o Domicilio:** ESTADOUNIDENSE **Lugar de Constitución:** ESTADOUNIDENSE **Teléfono:** _____ **Fax:** _____ **E-mail:** _____	**IDENTIFICACIÓN** **C.C.** [ ] **NIT** [ ] **C.E.** [ ] **Otro** [ ] **Cual** _____ **Número** _____
REPRESENTANTE APODERADO	**Nombre:** J. IAN RAISBECK **Dirección:** World Trade Center Torre A, Calle 100 No. 8A-37 Piso 10 **Teléfono:** 601-7700 **Fax:** 601-7799 **E-mail:** office@olarteraisbeck.com	**IDENTIFICACIÓN** **C.C.** [X] **NIT** [ ] **C.E.** [ ] **Otro** [ ] **Cual** _____ **Número** 79.376.996Bta **TP** 135.799
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(3) **PCT (86)**

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(6) **Título (54)** **PROTEINAS DE FUSION DE ALBUMINA**

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(10) **ANEX S**

- [x] Comprobante de pago de la tasa de presentación de la solicitud.
- [ ] Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.
- [ ] Poderes, si fuere el caso.
- [ ] Documento de cesión del inventor al solicitante o a su causante.
- [ ] Declaraciones.
- [x] Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos).
- [x] Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos).
- [ ] Copia del examen preliminar internacional efectuado por la Autoridad Internacional de Examen Preliminar (IPEA).
- [ ] Traducción del examen preliminar internacional efectuado por la IPEA.
- [ ] De ser el caso, copia del contrato de acceso.
- [ ] De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.
- [ ] De ser el caso, certificado de depósito del material biológico.
- [ ] Arte final 12 x 12.
- [ ] De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionadas parcial o totalmente con la invención de esta solicitud.
- [ ] De ser el caso, modificaciones efectuadas a la solicitud internacional.
- [x] Otros. Búsqueda Internacional

(11) **FIGURA CARACTERÍSTICA:**

(12) Solicito la concesión de la patente,

NOMBRE: J. IAN RAISBECK FIRMA:

C.C. 79.376.996 Bta T.P. 135.799

Instrucciones para el diligenciamiento del presente formulario, ver reverso de esta página.

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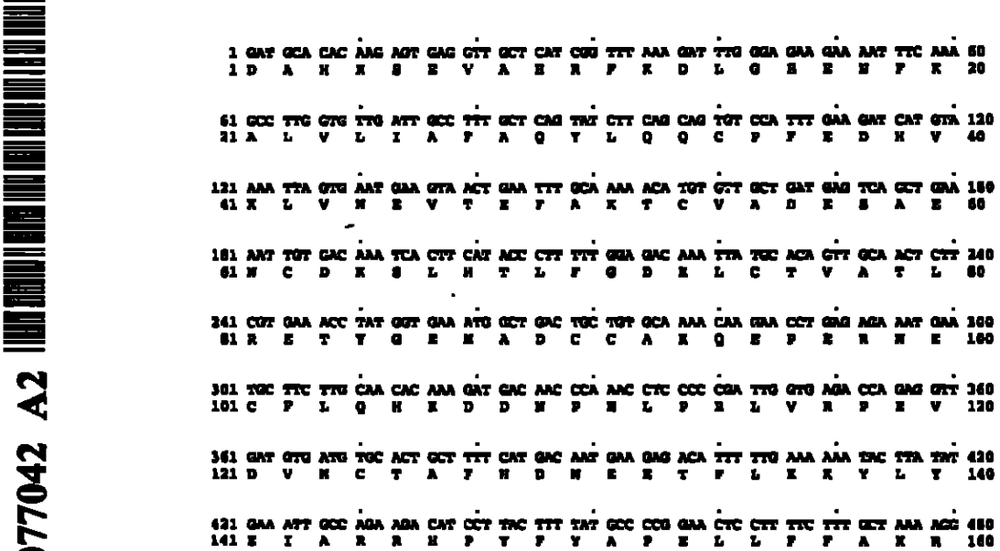
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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,

[Continued on next page]

(54) Title: ALBUMIN FUSION PROTEINS



(57) Abstract: The present invention encompasses albumin fusion proteins. Nucleic acid molecules encoding the albumin fusion proteins of the invention are also encompassed by the invention, as are vectors containing these nucleic acids, host cells transformed with these nucleic acids vectors, and methods of making the albumin fusion proteins of the invention and using these nucleic acids, vectors, and/or host cells. Additionally the present invention encompasses pharmaceutical compositions comprising albumin fusion proteins and methods of treating, preventing, or ameliorating diseases, disorders or conditions using albumin fusion proteins of the invention.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Albumin Fusion Proteins

REFERENCE TO SEQUENCE LISTING ON COMPACT DISC

[0001] This application refers to a "Sequence Listing" listed below, which is provided as an electronic document on three identical compact discs (CD-R), labeled "Copy 1," "Copy 2," and "Copy 3." These compact discs each contain the file "PF612PCT SL.txt" (929,048 bytes, created on February 7, 2005), which is incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] The invention relates generally to Therapeutic proteins (including, but not limited to, at least one polypeptide, antibody, peptide, or fragment and variant thereof) fused to albumin or fragments or variants of albumin. The invention encompasses polynucleotides encoding therapeutic albumin fusion proteins, therapeutic albumin fusion proteins, compositions, pharmaceutical compositions, formulations and kits. Host cells transformed with the polynucleotides encoding therapeutic albumin fusion proteins are also encompassed by the invention, as are methods of making the albumin fusion proteins of the invention using these polynucleotides, and/or host cells.

[0003] Human serum albumin (HSA, or HA), a protein of 585 amino acids in its mature form (as shown in Figure 1 (SEQ ID NO:1)), is responsible for a significant proportion of the osmotic pressure of serum and also functions as a carrier of endogenous and exogenous ligands. At present, HA for clinical use is produced by extraction from human blood. The production of recombinant HA (rHA) in microorganisms has been disclosed in EP 330 451 and EP 361 991.

[0004] Therapeutic proteins in their native state or when recombinantly produced, such as interferons and growth hormones, are typically labile molecules exhibiting short shelf-lives, particularly when formulated in aqueous solutions. The instability in these molecules when formulated for administration dictates that many of the molecules must be lyophilized and refrigerated at all times during storage, thereby rendering the molecules difficult to transport and/or store. Storage problems are particularly acute when pharmaceutical formulations must be stored and dispensed outside of the hospital environment.

[0005] Few practical solutions to the storage problems of labile protein molecules have been proposed. Accordingly, there is a need for stabilized, long lasting formulations of proteinaceous therapeutic molecules that are easily dispensed, preferably with a simple formulation requiring minimal post-storage manipulation.

[0006] Upon *in vivo* administration, therapeutic proteins in their native state or when recombinantly produced, such as interferons and growth hormones, exhibit a short plasma stability due to rapid clearance from the bloodstream. Accordingly, the therapeutic effects provided by these proteins are also short-lived. Thus, in order to sustain their desired therapeutic effect *in vivo*, the rapid clearance of these proteins from the blood dictates that the therapeutic molecules must be administered more frequently or at a higher dose. However, increasing the dosing schedule for administration of the therapeutic protein often results in an increase in injection site reactions, side-effects, and toxicity in the patient. Similarly, administration of the therapeutic protein at a higher dose also commonly results in an increase in toxicity and side-effects in the patient.

[0007] The few practical solutions to increasing plasma stability of therapeutic molecules that have been proposed, including chemical conjugation, have provided limited benefit to the patient. Generally, in most cases, these chemically modified therapeutic molecules are still administered on a frequent dosing schedule, retaining significant injection site reactions, side-effects, and toxicity in patients. Accordingly, there is a need for an stabilized form of therapeutic molecules that retains a higher plasma stability in vivo than the native or recombinantly produced therapeutic alone and can be administered less frequently, thereby decreasing potential side-effects to the patient.

SUMMARY OF THE INVENTION

[0008] The present invention encompasses albumin fusion proteins comprising a Therapeutic protein (e.g., a polypeptide, antibody, or peptide, or fragment or variant thereof) fused to albumin or a fragment (portion) or variant of albumin. The present invention also encompasses polynucleotides comprising, or alternatively consisting of, nucleic acid molecules encoding a Therapeutic protein (e.g., a polypeptide, antibody, or peptide, or fragment or variant thereof) fused to albumin or a fragment (portion) or variant of albumin. The present invention also encompasses polynucleotides, comprising, or alternatively consisting of, nucleic acid molecules encoding proteins comprising a Therapeutic protein (e.g., a polypeptide, antibody, or peptide, or fragment or variant thereof) fused to albumin or a fragment (portion) or variant of albumin, that is sufficient to prolong the shelf life of the Therapeutic protein, to increase the plasma stability of the Therapeutic protein compared to its unfused state, and/or stabilize the Therapeutic protein and/or its activity in solution (or in a pharmaceutical composition) *in vitro* and/or *in vivo*. Albumin fusion proteins encoded by a polynucleotide of the invention are also encompassed by the invention, as are host cells transformed with polynucleotides of the invention, and methods of making the albumin fusion proteins of the invention and using these polynucleotides of the invention, and/or host cells.

[0009] In a preferred aspect of the invention, albumin fusion proteins include, but are not limited to, those described in Table 2 and the polynucleotides encoding such proteins.

[0010] The invention also encompasses pharmaceutical formulations comprising an albumin fusion protein of the invention and a

pharmaceutically acceptable diluent or carrier. Such formulations may be in a kit or container. Such kit or container may be packaged with instructions pertaining to the extended shelf life of the Therapeutic protein. Such formulations may be used in methods of treating, preventing, ameliorating or diagnosing a disease or disease symptom in a patient, preferably a mammal, most preferably a human, comprising the step of administering the pharmaceutical formulation to the patient.

[0011] In other embodiments, the present invention encompasses methods of preventing, treating, or ameliorating a disease or disorder. In preferred embodiments, the present invention encompasses a method of treating a disease or disorder listed in the "Preferred Indication: Y" column of Table 1 comprising administering to a patient in which such treatment, prevention or amelioration is desired an albumin fusion protein of the invention that comprises a Therapeutic protein or portion corresponding to a Therapeutic protein (or fragment or variant thereof) disclosed in the "Therapeutic Protein: X" column of Table 1 (in the same row as the disease or disorder to be treated as listed in the "Preferred Indication: Y" column of Table 1) in an amount effective to treat, prevent or ameliorate the disease or disorder.

[0012] In one embodiment, an albumin fusion protein described in Table 1 or 2 has extended shelf life.

[0013] In a second embodiment, an albumin fusion protein described in Table 1 or 2 is more stable than the corresponding unfused Therapeutic molecule described in Table 1.

[0014] The present invention further includes transgenic organisms modified to contain the nucleic acid molecules of the invention (including, but not limited to, the polynucleotides described in Tables 1 and 2), preferably modified to express an albumin fusion protein of the invention.

BRIEF DESCRIPTION OF THE FIGURES

[0015] Figure 1A-D shows the amino acid sequence of the mature form of human albumin (SEQ ID NO:1) and a polynucleotide encoding it (SEQ ID NO:2). Nucleotides 1 to 1755 of SEQ ID NO:2 encode the mature form of human albumin (SEQ ID NO:1).

[0016] Figure 2 shows the restriction map of the pPPC0005 cloning vector ATCC deposit PTA-3278.

[0017] Figure 3 shows the restriction map of the pSAC35 yeast *S. cerevisiae* expression vector (Sleep *et al.*, BioTechnology 8:42 (1990)).

[0018] Figure 4 shows the effect of various dilutions of IFNb albumin fusion proteins encoded by DNA comprised in CID 2011 and 2053 on SEAP activity in the ISRE-SEAP/293F reporter cells (see Example 76). Proteins were serially diluted from 5e-7 to 1e-14 g/ml in DMEM/10% FBS and used to treat ISRE-SEAP/293F reporter cells. After 24 hours supernatants were removed from reporter cells and assayed for SEAP activity. IFNb albumin fusion protein was purified from three stable clones: 293F/#2011, CHO/#2011 and NSO/#2053. Mammalian derived IFNb, Avonex, came from Biogen and was reported to have a specific activity of 2.0e5 IU/ug.

[0019] Figure 5 compares the anti-proliferative activity of IFN albumin fusion protein encoded by CID 3165 (CID 3165 protein) and recombinant IFNa (rIFNa) on Hs294T melanoma cells. The cells were cultured with varying concentrations of either CID 3165 protein or rIFNa and proliferation was measured by BrdU incorporation after 3 days of culture. CID 3165 protein caused measurable inhibition of cell proliferation at concentrations above 10 ng/ml with 50% inhibition achieved at approximately 200 ng/ml. (■) = CID 3165 protein, (◆) = rIFNa.

[0020] Figure 6 shows the effect of various dilutions of IFNa albumin fusion proteins on SEAP activity in the ISRE-SEAP/293F reporter cells. One preparation of IFNa fused upstream of albumin (◆) was tested, as well as two different preparations of IFNa fused downstream of albumin (●) and (■).

[0021] Figure 7 shows the effect of time and dose of IFNa albumin fusion protein encoded by DNA comprised in construct Z249 (CID 2249 protein) on the mRNA level of OAS (p41) in treated monkeys (see Example 78). Per time point: first bar = Vehicle control, 2nd bar = 30 ug/kg CID 2249 protein day 1 iv, third bar = 30 ug/kg CID 2249 protein day 1 sc, 4th bar = 300 ug/kg CID Z249 protein day 1 sc, 5th bar = 40 ug/kg recombinant IFNa day 1, 3 and 5 sc.

[0022] Figure 8 shows the dose-response relationship of BNP albumin fusion proteins encoded by DNA comprised in constructs CID 3691 and 3618 (CID 3691 and 3618 protein) on activating cGMP formation in NPR-A/293F reporter cells (see Examples 80 and 81). Both recombinant BNP (■), as well as, two different preparations of BNP fused upstream of albumin (□) and (●) were tested.

[0023] Figure 9 shows the effect of BNP albumin fusion protein on mean arterial pressure in spontaneously hypertensive rats (see Example 80). Vehicle (□), recombinant BNP protein (●), or BNP albumin fusion protein (○) were delivered via tail vein injection. Systolic and diastolic blood pressures were recorded by cuff-tail method

[0024] Figure 10 shows the plasma cGMP levels in eleven- to 12-week-old male C57/BL6 mice after intravenous injection of recombinant BNP protein (●) or BNP albumin fusion protein (○ (see Example 80). cGMP levels were determined from plasma prepared from tail bleeds collected at several time points after intravenous injection.

[0025] Figure 11 shows the blood glucose levels in fasted ~8-week old diabetic db/db mice 24 hours after single administration of tandem GLP-1(7-36A8G)2x-HSA fusion (CID 3610) (◇), monomer GLP-1(7-36A8G)-HSA fusion (△), or HSA alone (●). Blood glucose levels were measured by an oral glucose tolerance test. Tandem GLP-1(7-36A8G)2x-HSA fusion (CID 3610) (◇) had an unexpected more potent glucose-normalizing activity than the monomer GLP-1(7-36A8G)-HSA fusion (△) 24 hours after a single administration in fasted ~8 week old diabetic db/db mice.

DETAILED DESCRIPTION

Definitions

[0026] The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

[0027] As used herein, "polynucleotide" refers to a nucleic acid molecule having a nucleotide sequence encoding a fusion protein comprising, or alternatively consisting of, at least one molecule of albumin (or a fragment or variant thereof) joined in frame to at least one Therapeutic protein X (or fragment or variant thereof); a nucleic acid molecule having a nucleotide sequence encoding a fusion protein comprising, or alternatively consisting of, the amino acid sequence of SEQ ID NO:Y (as described in column 6 of Table 2) or a fragment or variant thereof; a nucleic acid molecule having a nucleotide sequence comprising or alternatively consisting of the sequence shown in SEQ ID NO:X; a nucleic acid molecule having a nucleotide sequence encoding a fusion protein comprising, or alternatively consisting of, the amino acid sequence of SEQ ID NO:Z; a nucleic acid molecule having a nucleotide sequence encoding an albumin fusion protein of the invention generated as described in Table 2 or in the Examples; a nucleic acid molecule having a nucleotide sequence encoding a Therapeutic albumin fusion protein of the invention, a nucleic acid molecule having a nucleotide sequence contained in an albumin fusion construct described in Table 2, or a nucleic acid molecule having a nucleotide sequence contained in an albumin fusion construct deposited with the ATCC (as described in Table 3).

[0028] As used herein, "albumin fusion construct" refers to a nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide encoding at least one molecule of albumin (or a fragment or variant thereof) joined in frame to at least one polynucleotide encoding at least one molecule of a Therapeutic protein (or fragment or variant thereof); a nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide encoding at least one molecule of albumin (or a fragment or variant thereof) joined in frame to at least one polynucleotide encoding at least one molecule of a Therapeutic protein (or fragment or variant thereof) generated as described in Table 2 or in the Examples; or a nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide encoding at least one molecule of albumin (or a fragment or variant thereof) joined in frame to at least one polynucleotide encoding at least one molecule of a Therapeutic protein (or fragment or variant thereof), further comprising, for example, one or more of the following elements: (1) a functional self-replicating vector (including but not limited to, a shuttle vector, an expression vector, an integration vector, and/or a replication system), (2) a region for initiation of transcription (e.g., a promoter region, such as for example, a regulatable or inducible promoter, a constitutive promoter), (3) a region for termination of transcription, (4) a leader sequence, and (5) a selectable marker. The polynucleotide encoding the Therapeutic protein and albumin protein, once part of the albumin fusion construct, may each be referred to as a "portion," "region" or "moiety" of the albumin fusion construct.

[0029] The present invention relates generally to polynucleotides encoding albumin fusion proteins; albumin fusion proteins; and methods of treating, preventing, or ameliorating diseases or disorders using albumin fusion proteins or polynucleotides encoding albumin fusion proteins. As used herein, "albumin fusion protein" refers to a protein formed by the fusion of at least one molecule of albumin (or a fragment or variant thereof) to at least one molecule of a Therapeutic protein (or fragment or variant thereof). An albumin fusion protein of the invention comprises at least a fragment or variant of a Therapeutic protein and at least a fragment or variant of human serum albumin, which are associated with one another by genetic fusion (i.e., the albumin fusion protein is generated by translation of a nucleic acid in which a polynucleotide encoding all or a portion of a Therapeutic protein is joined in-frame with a polynucleotide encoding all or a portion of albumin). The Therapeutic protein and albumin protein, once part of the albumin fusion protein, may each be referred to as a "portion", "region" or "moiety" of the albumin fusion protein (e.g., a "Therapeutic protein portion" or an "albumin protein portion"). In a highly preferred embodiment, an albumin fusion protein of the invention comprises at least one molecule of a Therapeutic protein X or fragment or variant of thereof (including, but not limited to a mature form of the Therapeutic protein X) and at least one molecule of albumin or fragment or variant thereof (including but not limited to a mature form of albumin).

[0030] In a further preferred embodiment, an albumin fusion protein of the invention is processed by a host cell and secreted into the surrounding culture medium. Processing of the nascent albumin fusion protein that occurs in the secretory pathways of the host used for expression may include, but is not limited to signal peptide cleavage; formation of disulfide bonds; proper folding; addition and processing of carbohydrates (such as for example, N- and O- linked glycosylation); specific proteolytic cleavages; and assembly into multimeric proteins. An albumin fusion protein of the invention is preferably in the processed form. In a most preferred embodiment, the "processed form of an albumin fusion protein" refers to an albumin fusion protein product which has undergone N- terminal signal peptide cleavage, herein also referred to as a "mature albumin fusion protein".

[0031] In several instances, a representative clone containing an albumin fusion construct of the invention was deposited with the American Type Culture Collection (herein referred to as "ATCC®"). Furthermore, it is possible to retrieve a given albumin fusion construct from the deposit by techniques known in the art and described elsewhere herein. The ATCC® is located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC® deposits were made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure.

[0032] In one embodiment, the invention provides a polynucleotide encoding an albumin fusion protein comprising, or alternatively consisting of, a Therapeutic protein and a serum albumin protein. In a further embodiment, the invention provides an albumin fusion protein comprising, or

alternatively consisting of, a Therapeutic protein and a serum albumin protein. In a preferred embodiment, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a Therapeutic protein and a serum albumin protein encoded by a polynucleotide described in Table 2. In a further preferred embodiment, the invention provides a polynucleotide encoding an albumin fusion protein whose sequence is shown as SEQ ID NO:Y in Table 2. In other embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a biologically active and/or therapeutically active fragment of a Therapeutic protein and a serum albumin protein. In other embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a biologically active and/or therapeutically active variant of a Therapeutic protein and a serum albumin protein. In preferred embodiments, the serum albumin protein component of the albumin fusion protein is the mature portion of serum albumin. The invention further encompasses polynucleotides encoding these albumin fusion proteins.

[0033] In further embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a Therapeutic protein, and a biologically active and/or therapeutically active fragment of serum albumin. In further embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a Therapeutic protein and a biologically active and/or therapeutically active variant of serum albumin. In preferred embodiments, the Therapeutic protein portion of the albumin fusion protein is the mature portion of the Therapeutic protein. In a further preferred embodiment, the Therapeutic protein portion of the albumin fusion protein is the extracellular soluble domain of the Therapeutic protein. In an alternative embodiment, the Therapeutic protein portion of the albumin fusion protein is the active form of the Therapeutic protein. The invention further encompasses polynucleotides encoding these albumin fusion proteins.

[0034] In further embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a biologically active and/or therapeutically active fragment or variant of a Therapeutic protein and a biologically active and/or therapeutically active fragment or variant of serum albumin. In preferred embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, the mature portion of a Therapeutic protein and the mature portion of serum albumin. The invention further encompasses polynucleotides encoding these albumin fusion proteins.

Therapeutic protein

[0035] As stated above, a polynucleotide of the invention encodes a protein comprising or alternatively consisting of, at least a fragment or variant of a Therapeutic protein and at least a fragment or variant of human serum albumin, which are associated with one another, preferably by genetic fusion.

[0036] An additional embodiment includes a polynucleotide encoding a protein comprising or alternatively consisting of at least a fragment or variant of a Therapeutic protein and at least a fragment or variant of human serum albumin, which are linked with one another by chemical conjugation.

[0037] As used herein, "Therapeutic protein" refers to proteins, polypeptides, antibodies, peptides or fragments or variants thereof, having one or more therapeutic and/or biological activities. Therapeutic proteins encompassed by the invention include but are not limited to, proteins, polypeptides, peptides, antibodies, and biologics. (The terms peptides, proteins, and polypeptides are used interchangeably herein.) It is specifically contemplated that the term "Therapeutic protein" encompasses antibodies and fragments and variants thereof. Thus a protein of the invention may contain at least a fragment or variant of a Therapeutic protein, and/or at least a fragment or variant of an antibody. Additionally, the term "Therapeutic protein" may refer to the endogenous or naturally occurring correlate of a Therapeutic protein.

[0038] By a polypeptide displaying a "therapeutic activity" or a protein that is "therapeutically active" is meant a polypeptide that possesses one or more known biological and/or therapeutic activities associated with a therapeutic protein such as one or more of the Therapeutic proteins described herein or otherwise known in the art. As a non-limiting example, a "Therapeutic protein" is a protein that is useful to treat, prevent or ameliorate a disease, condition or disorder. As a non-limiting example, a "Therapeutic protein" may be one that binds specifically to a particular cell type (normal (e.g., lymphocytes) or abnormal e.g., (cancer cells)) and therefore may be used to target a compound (drug, or cytotoxic agent) to that cell type specifically.

[0039] For example, a non-exhaustive list of "Therapeutic protein" portions which may be comprised by an albumin fusion protein of the invention includes, but is not limited to, GLP-1, GLP-2, PACAP-27, PACAP-38, VIP, CD4M33, secretin, glicentin, oxyntomodulin, PHM, IFNα, IFNβ, ANP, BNP, NGF, BDNF, GDNF, and somatostatin.

[0040] Interferon hybrids may also be fused to the amino or carboxy terminus of albumin to form an interferon hybrid albumin fusion protein. Interferon hybrid albumin fusion protein may have enhanced, or alternatively, suppressed interferon activity, such as antiviral responses, regulation of cell growth, and modulation of immune response (Leblau et al., *PNAS* USA, 73:3107-3111 (1976); Gresser et al., *Nature*, 251:543-545 (1974); and Johnson, *Texas Reports Biol Med*, 35:357-369 (1977)). Each interferon hybrid albumin fusion protein can be used to treat, prevent, or ameliorate viral infections (e.g., hepatitis (e.g., HCV); or HIV), multiple sclerosis, or cancer.

[0041] In one embodiment, the interferon hybrid portion of the interferon hybrid albumin fusion protein comprises an interferon alpha-interferon alpha hybrid (herein referred to as an alpha-alpha hybrid). For example, the alpha-alpha hybrid portion of the interferon hybrid albumin fusion protein consists, or alternatively comprises, of interferon alpha A fused to interferon alpha D. In a further embodiment, the A/D hybrid is fused at the common BglII restriction site to interferon alpha D, wherein the N-terminal portion of the A/D hybrid corresponds to amino acids 1-62 of

interferon alpha A and the C-terminal portion corresponds to amino acids 64-166 of interferon alpha D. For example, this A/D hybrid would comprise the amino acid sequence:

CDLPQTHSLGSRRTLMLLAQMRX$_1$ISLFSCLKDRHDFGFPQEEFGNQFQKAETIPVLHEMIQQIFNLFTTKDSSAAWDEDLLDKFCTELYQQL NDLEACVMQEERVGETPLMNX$_2$DSILAVKKYFRRITLYLTEKKYSPCAWEVVRAEIMRSLSLSTNLQERLRRKE (SEQ ID NO:99), wherein the X_1 is R or K and the X_2 is A or V (see, for example, Construct ID #2875). In an additional embodiment, the A/D hybrid is fused at the common PvuII restriction site, wherein the N-terminal portion of the A/D hybrid corresponds to amino acids 1-91 of interferon alpha A and the C-terminal portion corresponds to amino acids 93-166 of interferon alpha D. For example, this A/D hybrid would comprise the amino acid sequence:

CDLPQTHSLGSRRTLMLLAQMRX$_1$ISLFSCLKDRHDFGFPQEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQL NDLEACVMQEERVGETPLMNX$_2$DSILAVKKYFRRITLYLTEKKYSPCAWEVVRAEIMRSLSLSTNLQERLRRKE (SEQ ID NO:100), wherein the X_1 is R or K and the second X_2 is A or V (see, for example, Construct ID #2872). These hybrids are further described in U.S. Patent No. 4,414,510, which is hereby incorporated by reference in its entirety.

[0042] In an additional embodiment, the alpha-alpha hybrid portion of the interferon hybrid albumin fusion protein consists, or alternatively comprises, of interferon alpha A fused to interferon alpha F. In a further embodiment, the A/F hybrid is fused at the common PvuII restriction site, wherein the N-terminal portion of the A/F hybrid corresponds to amino acids 1-91 of interferon alpha A and the C-terminal portion corresponds to amino acids 93-166 of interferon alpha F. For example, this A/F hybrid would comprise the amino acid sequence:

CDLPQTHSLGSRRTLMLLAQMRXISLFSCLKDRHDFGFPQEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQL NDMEACVIQEVGVEETPLMNVDSILAVKKYFQRITLYLTEKKYSPCAWEVVRAEIMRSFSLSKIFQERLRRKE (SEQ ID NO:101), wherein X is either R or K (see, for example, Construct ID #2874). These hybrids are further described in U.S. Patent No. 4,414,510, which is hereby incorporated by reference in its entirety. In a further embodiment, the alpha-alpha hybrid portion of the interferon hybrid albumin fusion protein consists, or alternatively comprises, of interferon alpha A fused to interferon alpha B. In an additional embodiment, the A/B hybrid is fused at the common PvuII restriction site, wherein the N-terminal portion of the A/B hybrid corresponds to amino acids 1-91 of interferon alpha A and the C-terminal portion corresponds to amino acids 93-166 of interferon alpha B. For example, this A/B hybrid would comprise an amino acid sequence:

CDLPQTHSLGSRRTLMLLAQMRX$_1$ISLFSCLKDRHDFGFPQEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQL NDLEX$_2$X$_3$X$_4$X$_5$QEVGVIESPLMYEDSILAVRKYFQRITLYLTEKKYSSCAWEVVRAEIMRSFSLSTNLQKRLKSKE (SEQ ID NO:102), wherein the X_1 is R or K and X_2 through X_5 is SCVM or VLCD (see, for example, Construct ID #2873). These hybrids are further described in U.S. Patent No. 4,414,510, which is hereby incorporated by reference in its entirety.

[0043] In another embodiment, the interferon hybrid portion of the interferon hybrid albumin fusion protein comprises an interferon beta-interferon alpha hybrid (herein referred to as a beta-alpha hybrid). For example, the beta-alpha hybrid portion of the interferon hybrid albumin fusion protein consists, or alternatively comprises, of interferon beta-1 fused to interferon alpha D (also referred to as interferon alpha-1). In a further embodiment, the beta-1/alpha D hybrid is fused wherein the N-terminal portion corresponds to amino acids 1-73 of interferon beta-1 and the C-terminal portion corresponds to amino acids 74-167 of interferon alpha D. For example, this beta-1/alpha D hybrid would comprise an amino acid sequence:

MSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTIYEMLQNIFAIFRQDSSAAWDEDLLDKFCTELY QQLNDLEACVMQEERVGETPLMNXDSILAVKKYFRRITLYLTEKKYSPCAWEVVRAEIMRSLSLSTNLQERLRRKE (SEQ ID NO:103), wherein X is A or V. These hybrids are further described in U.S. Patent No. 4,758,428, which is hereby incorporated by reference in its entirety.

[0044] In another embodiment, the interferon hybrid portion of the interferon hybrid albumin fusion protein comprises an interferon alpha-interferon beta hybrid (herein referred to as a alpha-beta hybrid). For example, the alpha-beta hybrid portion of the interferon hybrid albumin fusion protein consists, or alternatively comprises, of interferon alpha D (also referred to as interferon alpha-1) fused to interferon beta-1. In a further embodiment, the alpha D/beta-1 hybrid is fused wherein the N-terminal portion corresponds to amino acids 1-73 of interferon alpha D and the C-terminal portion corresponds to amino acids 74-166 of interferon beta-1. For example, this alpha D/beta-1 hybrid would have an amino acid sequence:

MCDLPETHSLDNRRTLMLLAQMSRISPSSCLMDRHDFGFPQEEFDGNQFQKAPAISVLHELIQQIFNLFTTKDSSSTGWNETIVENLLANVY HQINHLKTVLEEKLEKEDFTRGKLMSSLHLKRYYGRILHYLKAKEYSHCAWTIVRVEILRNFYFINRLTGYLRN (SEQ ID NO:104). These hybrids are further described in U.S. Patent No. 4,758,428, which is hereby incorporated by reference in its entirety.

[0045] In further embodiments, the interferon hybrid portion of the interferon hybrid albumin fusion proteins may comprise additional combinations of alpha-alpha interferon hybrids, alpha-beta interferon hybrids, and beta-alpha interferon hybrids. In additional embodiments, the interferon hybrid portion of the interferon hybrid albumin fusion protein may be modified to include mutations, substitutions, deletions, or additions to the amino acid sequence of the interferon hybrid. Such modifications to the interferon hybrid albumin fusion proteins may be made, for example, to improve levels of production, increase stability, increase or decrease activity, or confer new biological properties.

[0046] The above-described interferon hybrid albumin fusion proteins are encompassed by the invention, as are host cells and vectors containing polynucleotides encoding the polypeptides. In one embodiment, a interferon hybrid albumin fusion protein encoded by a polynucleotide as

described above has extended shelf life. In an additional embodiment, a interferon hybrid albumin fusion protein encoded by a polynucleotide described above has a longer serum half-life and/or more stabilized activity in solution (or in a pharmaceutical composition) *in vitro* and/or *in vivo* than the corresponding unfused interferon hybrid molecule.

[0047] In another non-limiting example, a "Therapeutic protein" is a protein that has a biological activity, and in particular, a biological activity that is useful for treating, preventing or ameliorating a disease. A non-inclusive list of biological activities that may be possessed by a Therapeutic protein includes, inhibition of HIV-1 infection of cells, stimulation of intestinal epithelial cell proliferation, reducing intestinal epithelial cell permeability, stimulating insulin secretion, induction of bronchodilation and vasodilation, inhibition of aldosterone and renin secretion, blood pressure regulation, promoting neuronal growth, enhancing an immune response, enhancing inflammation, suppression of appetite, or any one or more of the biological activities described in the "Biological Activities" section below and/or as disclosed for a given Therapeutic protein in Table 1 (column 2).

[0048] In one embodiment, IFN-beta-HSA fusions are used to inhibit the activity of Ebola virus and the SARS virus (Toronto-2 strain). For example, the in vitro antiviral activity of IFN-beta fused upstream of mature HSA (CID 2053 protein) was evaluated against Ebola virus and SARS virus in Vero cells. These cells were used to assess the protective effects of CID 2053 protein based on inhibition of cytopathic effect (CPE) and the neutral red assay of cell viability. In vitro signal transduction was assessed by analysis of gene expression. Further, the pharmacokinetics and pharmacodynamics of CID 2053 protein were evaluated in rhesus monkeys. The results indicate that potent in vitro antiviral activity was achieved with a favorable safety index. The IC50 for CID 2053 protein was 0.4 ng/ml against Ebola and 2 ng/ml against the SARS virus. Array analysis showed that CID 2053 protein and IFN-beta induce the expression of a similar set of genes and trigger the IFN-stimulated response element (ISRE) signal transduction pathway. In rhesus monkeys administered a dose of 50 ug/kg IV or SC or 300 ug/kg SC CID 2053 protein, the terminal half-life was 36-40 hours. Administration of CID 2053 protein induced sustained increases in serum neopterin levels and OAS1 mRNA expression.

[0049] In a further embodiment, IFN-alpha-HSA fusions are used to inhibit viral agents classified under Category A- Filo (Ebola), Arena (Pichende), Category B- Toga (VEE) or Category C- Bunya (Punto toro), Flavi (Yellow fever, West Nile). For example, CPE inhibition, neutral red staining and virus yield assays were employed to evaluate the antiviral activities of INF-alpha fused downstream of HSA (CID 3165 protein). The pharmacokinetics and pharmacodynamic activity of CID 3165 protein in cynomolgus monkeys and human subjects were evaluated. The results indicate that antiviral activity was achieved against all the RNA viruses evaluated with a favorable safety index. The IC50 values ranged from <0.1 ng/ml (Punta Toro A) to 19 ng/ml (VEE) in the CPE assay. In cynomolgus monkeys, the half-life of CID 3165 protein was 90 hours and was detectable up to 14 days post-dose. In human subjects, CID 3165 protein was safe and well tolerated. C_{max} following single injection doses was dose-proportional. The mean C_{max} in the 500 ug cohort was 22 ng/ml, and the mean $t_{1/2}$ was 150 hours. Dosing once every 2-4 weeks or more is supported by the pharmacokinetics. Antiviral response against Hepatitis C was observed in the majority of subjects in the single injection cohorts (120-500 ug).

[0050] In a further embodiment, IFN-alpha-HSA fusions are used to treat patients with chronic Hepatitis C infection (HCV). Interferon alpha, also known as interferon alfa or leukocyte inferon, is the standard of care for treatment of patients infected with HCV. The term "interferon alpha" refers to a family of highly homologous related polypeptides with anti-viral activity. The interferon alpha portion of the IFN-alpha-HSA fusion consists or alternatively comprises any interferon alpha or fragment thereof known in the art. Non-limiting examples of interferon alpha encompassed by the invention include, but are not limited to, the interferon alpha proteins disclosed in the Therapeutic protein column of Table 1. In particular embodiments, the interferon alpha portion consists or alternatively comprises interferon alpha-2a, interferon alpha-2b, interferon alpha-2c, consensus interferon, interferon alfacon-1, interferon alpha-n1, interferon alpha-n3, any commercially available form of interferon alpha, such as, for example, INTRON® A (Schering Corp., Kenilworth, N.J.), ROFERON® A (Hoffman-La Roche, Nutley, N.J.), Berofor alpha interferon (Boehringer Ingelheim Pharmaceutical, Inc., Ridgefied, Conn.), OMNIFERON™ (Viragen, Inc., Plantation, FL), MULTIFERON™ (Viragen, Inc., Plantation, FL) WELLFERON® (GlaxoSmithKline, London, Great Britian), INFERGEN® (Amgen, Inc., Thousands Oaks, CA), SUMIFERON® (Sumitomo, Japan), BELEROFON® (Nautilus Biotech, France) or any purified interferon alpha product or a fragment thereof. In additional embodiments, the interferon alpha portion of the IFN-alpha-HSA fusion protein may be modified by the attachment of chemical moieties. For example, the interferon alpha portion may be modified by pegylation. Accordingly, in additional embodiments, the interferon alpha portion of the IFN-alpha-HSA fusion protein consists or alternatively comprises pegylated forms of interferon alpha-2a, 2b, or consensus interferon and include, but are not limited to, a commercially available pegylated interferon alpha, such as, for example, PEG-INTRON® (Schering Corp., Kenilworth, N.J.), PEGASYS® (Hoffman-La Roche, Nutley, N.J.), PEG-OMNIFERON™ (Viragen, Inc., Plantation, FL) or a fragment thereof. However, as used herein, "IFN-alpha-HSA" fusions refers to the HSA fused to any of the interferon alpha proteins known in the art or a fragment thereof.

[0051] Patients infected with HCV may fall within two categories based on previous exposure to an interferon regimen for treatment of the HCV infection. "Naive patients" are those patients who have never been treated with an interferon regimen. "Experienced patients" are those patients who have been treated or are currently being treated with an interferon regimen. "Non-responders" are experienced patients who have been previously treated with an interferon regimen but have failed to meet the primary endpoint of treatment such as an early viral load reduction (EVR) or an end-of-treatment response (ETR). However, as used herein, an "HCV patient" refers to a patient who is infected with HCV and who is either

naive, experienced, or a non-responder.

[0052] In addition, the Hepatitis C virus can be classified into four genotypes, genotype 1, 2, 3, or 4. Generally, the Hepatitis C virus that infects an HCV patient comprises a single genotype. However, the Hepatitis virus can comprise a combination of two or more genotypes. In addition, the genotype of Hepatitis C virus may also be a variant of one of the known HCV genotypes. In a further embodiment, the Hepatitis C virus of the HCV patient is genotype 1 or a variant thereof. However, as used herein, "HCV" refers to the Hepatitis C virus of any genotype, or combination or variants thereof.

[0053] The standard treatment regimen for patients with HCV involves treatment *with interferon alpha in combination with an antiviral agent*, such as, ribavirin. In general, the interferon alpha is administered daily, twice-a-week, or weekly and the ribavirin is administered daily. However, recent studies have also used interferon alpha in combination with other antiviral agents known in the art for the treatment of HCV. Thus, in a further embodiment the IFN-alpha-HSA fusion may be administered to the HCV patient either alone or in combination with an antiviral agent, such as, for example, ribavirin.

[0054] As noted above, pharmokinetics of the CID 3165 protein support a dosing schedule of once every 2-4 weeks or greater. Thus, in a further embodiment, the HCV patients are treated with an IFN-alpha-HSA fusion by administration once every 2-4 weeks alone or in combination with an effective amount of an antiviral agent. In a preferred embodiment, the HCV patients are treated with an IFN-alpha-HSA fusion by administration once every 2-4 weeks in combination with an effective amount of an antiviral agent. In an additional preferred embodiment, the IFN-alpha-HSA fusion is administered to the HCV patient once every 4 weeks. In an additional preferred embodiment, the IFN-alpha-HSA fusion is administered to the HCV patient more than once every 4 weeks. In additional embodiments, the IFN-alpha-HSA fusion is administered once every 4 weeks or more to an HCV patient, wherein the treatment also includes administration of an effective amount of an antiviral agent.

[0055] In another embodiment, IFN-alpha-HSA fusions may be used as a low-dose monotherapy for maintenance therapy of HCV. In a further additional embodiment, IFN-alpha-HSA fusions may used in combination with ribavirin and one or more other antiviral agents for the treatment of HCV. Alternatively, in another embodiment, IFN-alpha-HSA fusions may be used in combination with one or more antiviral agents, other than ribavirin, for the treatment of HCV.

[0056] In an additional embodiment, IFN-alpha-HSA fusions may be used for the treatment of other viral infections. For example, in one embodiment, IFN-alpha-HSA fusions may be used for the treatment of Hepatitis B (HBV). In an additional embodiment, IFN-alpha-HSA fusions may be used for the treatment of Human Papilloma Virus (HPV). In a further embodiment, IFN-alpha-HSA fusions may be used in the treatment of cancer, including, but not limited to hairy cell leukemia, malignant melanoma, follicular lymphoma, chronic myelogenous leukemia, AIDS related Kaposi's Sarcoma, multiple myeloma, or renal cell cancer.

[0057] In another embodiment, GLP-1-HSA fusions are used to regulate blood glucose levels in diabetic patients. In a further embodiment, tandem fusions of wild-type or mutant GLP-1 are used to regulate blood glucose levels in diabetic patients. For example, the ability of monomer *GLP-1(7-36ABG)-HSA and tandem GLP-1(7-36ABG)-HSA (CID 3610)* fusions to regulate the blood glucose levels were evaluated utilizing an oral glucose tolerance test (1 gram glucose/kg by oral gavage), following subcutaneous injection of GLP-1-HSA protein in ~8 week old diabetic db/db mice. This glucose tolerance test consisted of the subcutaneous injection of a GLP-1-HSA fusion followed by administration of 1 gram glucose/kg by oral gavage. Fasted diabetic db/db mice were administered equimolar doses (100 and 171 nmol/kg) of either the monomer or tandem GLP-1-HSA fusion protein and oral glucose tolerance tests were performed 6 or 24 hours after a single administration. Quite surprisingly and unexpected, the tandem GLP-1(7-36ABG)-HSA fusion (CID 3610) significantly reduced blood glucose at 6 hours after injection when compared to the monomer GLP-1(7-36ABG)-HSA fusion. In addition, the difference between monomer GLP-1(7-36ABG)-HSA and tandem GLP-1(7-36ABG)-HSA (CID 3610) fusions were even more dramatic when diabetic *db/db* mice were evaluated 24 hours after injection. As shown in Figure 11, the tandem GLP-1(7-36ABG)-HSA fusion (CID 3610) (◇) possessed a unexpectedly potent glucose-normalizing activity while fasting blood glucose levels for the single GLP-1(7-36ABG)-HSA (△) fusion was similar to animals administered HSA alone (●) and clearly were diabetic in nature.

[0058] As used herein, "therapeutic activity" or "activity" may refer to an activity whose effect is consistent with a desirable therapeutic outcome in humans, or to desired effects in non-human mammals or in other species or organisms. Therapeutic activity may be measured *in vivo* or *in vitro*. For example, a desirable effect may be assayed in cell culture. Such *in vitro* or cell culture assays are commonly available for many Therapeutic proteins as described in the art. Examples of assays include, but are not limited to those described herein in the Examples section or in the "Exemplary Activity Assay" column (column 3) of Table 1.

[0059] Therapeutic proteins corresponding to a Therapeutic protein portion of an albumin fusion protein of the invention, such as cell surface and secretory proteins, are often modified by the attachment of one or more oligosaccharide groups. The modification, referred to as glycosylation, can dramatically affect the physical properties of proteins and can be important in protein stability, secretion, and localization. Glycosylation occurs at specific locations along the polypeptide backbone. There are usually two major types of glycosylation: glycosylation characterized by O-linked oligosaccharides, which are attached to serine or threonine residues; and glycosylation characterized by N-linked oligosaccharides, which are attached to asparagine residues in an Asn-X-Ser or Asn-X-Thr sequence, where X can be any amino acid except proline. N-acetylneuraminic acid (also known as sialic acid) is usually the terminal residue of both N-linked and O-linked oligosaccharides. Variables such as protein structure and

cell type influence the number and nature of the carbohydrate units within the chains at different glycosylation sites. Glycosylation isomers are also common at the same site within a given cell type.

[0060] Therapeutic proteins corresponding to a Therapeutic protein portion of an albumin fusion protein of the invention, as well as analogs and variants thereof, may be modified so that glycosylation at one or more sites is altered as a result of manipulation(s) of their nucleic acid sequence, by the host cell in which they are expressed, or due to other conditions of their expression. For example, glycosylation isomers may be produced by abolishing or introducing glycosylation sites, e.g., by substitution or deletion of amino acid residues, such as substitution of glutamine for asparagine, or unglycosylated recombinant proteins may be produced by expressing the proteins in host cells that will not glycosylate them, e.g. in *E. coli* or glycosylation-deficient yeast. These approaches are described in more detail below and are known in the art.

[0061] Therapeutic proteins, particularly those disclosed in Table 1, and their nucleic acid and amino acid sequences are well known in the art and available in public databases such as Chemical Abstracts Services Databases (e.g., the CAS Registry), GenBank, and subscription provided databases such as GenSeq (e.g., Derwent). Exemplary nucleotide sequences of Therapeutic proteins which may be used to derive a polynucleotide of the invention are shown in column 7, "SEQ ID NO:X," of Table 2. Sequences shown as SEQ ID NO:X may be a wild type polynucleotide sequence encoding a given Therapeutic protein (e.g., either full length or mature), or in some instances the sequence may be a variant of said wild type polynucleotide sequence (e.g., a polynucleotide which encodes the wild type Therapeutic protein, wherein the DNA sequence of said polynucleotide has been optimized, for example, for expression in a particular species; or a polynucleotide encoding a variant of the wild type Therapeutic protein (i.e., a site directed mutant; an allelic variant)). It is well within the ability of the skilled artisan to use the sequence shown as SEQ ID NO:X to derive the construct described in the same row. For example, if SEQ ID NO:X corresponds to a full length protein, but only a portion of that protein is used to generate the specific CID, it is within the skill of the art to rely on molecular biology techniques, such as PCR, to amplify the specific fragment and clone it into the appropriate vector.

[0062] Additional Therapeutic proteins corresponding to a Therapeutic protein portion of an albumin fusion protein of the invention include, but are not limited to, one or more of the Therapeutic proteins or peptides disclosed in the "Therapeutic Protein X" column of Table 1 (column 1), or fragment or variable thereof.

[0063] Table 1 provides a non-exhaustive list of Therapeutic proteins that correspond to a Therapeutic protein portion of an albumin fusion protein of the invention, or an albumin fusion protein encoded by a polynucleotide of the invention. The first column, "Therapeutic Protein X," discloses Therapeutic protein molecules that may be followed by parentheses containing scientific and brand names of proteins that comprise, or alternatively consist of, that Therapeutic protein molecule or a fragment or variant thereof. "Therapeutic protein X" as used herein may refer either to an individual Therapeutic protein molecule, or to the entire group of Therapeutic proteins associated with a given Therapeutic protein molecule disclosed in this column. The "Biological activity" column (column 2) describes Biological activities associated with the Therapeutic protein molecule. Column 3, "Exemplary Activity Assay," provides references that describe assays which may be used to test the therapeutic and/or biological activity of a Therapeutic protein:X or an albumin fusion protein comprising a Therapeutic protein X (or fragment thereof) portion. Each of the references cited in the "Exemplary Activity Assay" column are herein incorporated by reference in their entireties, particularly with respect to the description of the respective activity assay described in the reference (see Methods section therein, for example) for assaying the corresponding biological activity set forth in the "Biological Activity" column of Table 1. The fourth column, "Preferred Indication: Y," describes disease, disorders, and/or conditions that may be treated, prevented, diagnosed, and/or ameliorated by Therapeutic protein X or an albumin fusion protein comprising a Therapeutic protein X (or fragment thereof) portion. The "Construct ID" column (column 5) provides a link to an exemplary albumin fusion construct disclosed in Table 2 which encodes an albumin fusion protein comprising, or alternatively consisting of the referenced Therapeutic Protein X (or fragment thereof) portion.

Table 1

Therapeutic Protein-X	Biological Activity	Exemplary Activity Assay	Preferred Indication Y	Construct ID	Therapeutic Protein-Z
Human growth hormone (Pegvisomant, Somatrem Somatropin TROVERT PROTROPIN BIO-TROPIN HUMATROPE, NUTROPIN NUTROPIN AQ NUTROPHIN NORDITROPIN GENOTROPIN SAIZEN SEROSTIM)	Binds to two GHR molecules and induces signal transduction through receptor dimerization	Ba/F3-hGHR proliferation assay a novel specific bioassay for serum human growth hormone. J Clin Endocrinol Metab 2000 Nov;85(11):4274-9 Plasma growth hormone (GH) immunoassay and tibial bioassay Appl Physiol 2000 Dec;89(6):2174-8 Growth hormone (hGH) receptor mediated cell mediated proliferation, Growth Horm IGF Res 2000 Oct,10(5):248-55 International standard for growth hormone, Horm Res 1999;51 Suppl 1-7 12	Acromegaly- Growth failure; Growth hormone replacement, Growth hormone deficiency- Pediatric Growth Hormone Deficiency- Adult Growth Hormone Deficiency- Idiopathic Growth Hormone Deficiency- Growth retardation- Prader-Willi Syndrome; Prader-Willi Syndrome in children 2 years or older- Growth deficiencies, Growth failure associated with chronic renal insufficiency- Osteoporosis Postmenopausal osteoporosis Osteoporosis, Osteochondrogenesis; burns, Cachexia, Cancer Cachexia, Dwarfism Metabolic Disorders; Obesity- Renal failure; Turner's Syndrome; Fibromyalgia; Fracture treatment; Frailty AIDS wasting; Muscle Wasting; Short Stature; Diagnostic Agents, Female Infertility; Hypodystrophy	3468, 3469 3470, 3475	See Table 2 SEQ ID NO:Z for particular construct.
Interferon alpha (Interferon alfa-2b; recombinant; Interferon alfa-n1 Interferon alfa-n3 Peginterferon alpha-2b; Ribavirin and interferon alfa-2b; Interferon alfacon-1 interferon consensus, YM 643 CIFN interferon -alpha consensus; recombinant methionyl consensus interferon; recombinant consensus interferon CGP 35269- RO 253034; RO 258310- INTRON A PEG-INTRON OIF OMNIFERON PEG-OMNIFERON VELDONA PEG-REBETRON ROFERON A, WELLFERON ALFERON N/LDO, REBETRON ALTEMOL, VIRAFERONPEG PEGASYS VIRAFERON VIRAFON AMPLIGEN INFERGEN INFAREX, ORAGEN)	Confers a range of cellular responses including antiviral, antiproliferative, antitumor and immunomodulatory activities; stimulate production of two enzymes, a protein kinase and an oligoadenylate synthetase.	Anti-viral assay- Rubinstein S Familletti PC, Pestka S. (1981) Convenient assay for interferons. J Virol. 37(2):755-8, Anti-proliferation assay- Gao Y et al (1999) Sensitivity of an epstein-barr virus-positive tumor line, Daudi, to alpha interferon correlates with expression of a GC-rich viral transcript. Mol Cell Biol 19(11)-7303-13.	Viral Infections include Severe Acute Respiratory Syndrome (SARS) and other coronavirus infections filoviruses, including but not limited to Ebola viruses and Marburg virus, Arenaviruses, including but not limited to Pichande virus, Lassa virus, Junin virus, Machupo virus, Guanarito virus, and lymphocytic choriomeningitis virus (LCMV), Bunyaviruses, including but not limited to Punta toro virus, Crimean-Congo hemorrhagic fever virus, sandfly fever viruses, Rift Valley fever virus, La Crosse virus and hantaviruses- Flaviviruses, including but not limited to Yellow Fever Banzi virus, West Nile virus, Dengue viruses, Japanese Encephalitis virus, Tick-borne encephalitis, Omsk Hemorrhagic Fever and Kyasanur Forest Disease virus Togaviruses, including but not limited to Venezuelan, eastern, and western equine encephalitis viruses, Ross River virus, and Rubella virus Orthopox viruses, including but not limited to Vaccina, Cowpox, Smallpox, and Monkeypox Herpesviruses, HuA/B; Respiratory Syncytial virus (RSV), parafiu; measles; rhinoviruses, adenoviruses, Semliki Forest virus Viral Hemorrhagic fevers, Rhabdoviruses; Paramyxoviruses, including but not limited to Nipah virus and Hendra virus, and other viral agents identified by the U.S. Centers for Disease Control and Prevention as high-priority disease agents (*i.e.* Category A, B, and C agents; see, *e.g.* Moran, Emerg. Med. Clin. North. Am 2002, 20(2):311-30	2249 2343, 2366, 2381 2382 2410, 3165, 3422, 3423 3424, 3476.	See Table 2, SEQ ID NO:Z for particular construct.

Table 1

Therapeutic Protein X	Biological Activity	Exemplary Activity Assay	Preferred Indication:Y	Construct ID	Therapeutic Protein Z
			and Daring et al Emerg. Med. Clin North Am 2002;20(2):273-309).		
Interferon beta (interferon beta-1a, interferon beta 1b; interferon-beta-serine; SH 579-ZK 157046, BCDF beta-2 IF interferon-beta-2 rhIL-6; SJ0031 DL 8234; FERON IFNbeta, BETASERON AVONEX REBIF-BETAFERON SIOSOIX)	Modulates MHC antigen expression, NK cell activity and IFNg production and IL12 production in monocytes.	Anti-viral assay- Rubinstein S, Familletti PC, Pestka S. (1981) Convenient assay for interferons. J Virol. 37(2):755-8 Anti-proliferation assay- Gao Y et al (1999) Sensitivity of an epstein-barr virus-positive tumor line, Daudi, to alpha interferon correlates with expression of a GC-rich viral transcript. Mol Cell Biol. 19(11):7305-13	Viral infections include Severe Acute Respiratory Syndrome (SARS) and other coronavirus infections, filoviruses, including but not limited to Ebola viruses and Marburg virus, Arenaviruses, including but not limited to Pichende virus, Lassa virus, Junin virus, Machupo virus, Guanarito virus and lymphocytic choriomeningitis virus (LCMV); Bunyaviruses, including but not limited to Punta toro virus, Crimean-Congo hemorrhagic fever virus, sandfly fever viruses, Rift Valley fever virus, La Crosse virus, and hantaviruses; Flavivirues, including but not limited to Yellow Fever Banzi virus, West Nile virus, Dengue viruses, Japanese Encephalitis virus, Tick-borne encephalitis, Omsk Hemorrhagic Fever and Kyasanur Forest Disease virus, Togaviruses, including but not limited to Venezuelan, eastern, and western equine encephalitis viruses, Ross River virus, and Rubella virus; Orthopox viruses, including but not limited to Vaccinia, Cowpox, Smallpox, and Monkeypox; Herpesviruses, FluA/B Respiratory Syncytial virus (RSV); parafluz; measles; rhinoviruses, adenoviruses; Semliki Forest virus, Viral Hemorrhagic fevers; Rhabdoviruses, Paramyxoviruses, including but not limited to Nipah virus and Hendra virus, and other viral agents identified by the U.S. Centers for Disease Control and Prevention as high-priority disease agents (i.e. Category A, B, and C agents; see, e.g. Moran, Emerg. Med. Clin. North. Am 2002 20(2):311-30 and Daring et al. Emerg. Med. Clin. North Am 2002,20(2):273-309).	1778, 1779 2011 2013, 2053, 2054, 2492, 2580, 2795 2796, 2797	See Table 2, SEQ ID NO:Z for particular construct.
B-type natriuretic peptide (BNP brain natriuretic peptide)	stimulates smooth muscle relaxation and vasodilation, natriuresis,and suppression of renin-angiotensin and endothelin.	Inhibition of angiotensin can be determined using assays known in the art, for example using an in vitro proliferation assay with rat cardiac fibroblasts as described in Naunyn Schmiedebergs Arch Pharmacol 1999 May;359(5):394-9. Vasodilation can be measured in animals by measuring the	Congestive heart failure; cardiac volume overload cardiac decompensation, Cardiac Failure; Left Ventricular Dysfunction, Dyspnea. Glomerular hypertrophy- Glomerular injury- Renal glomerular disease Acute Renal Failure.	3618, 3690, 3691 3715, 3723, 3724, 3725, 3736, 3769, 3778, 3783, 3795, 3796, 3809,	See Table 2 SEQ ID NO:Z for particular construct.

Table 1

Therapeutic Protein X	Biological Activity	Exemplary Activity Assay	Preferred Indication:Y	Construct ID	Therapeutic Protein Z
		myogenic responses of small renal arteries in an isobaric arteriograph system (see Am J Physiol Regul Integr Comp Physiol 2002 Aug;283(2):R349-R355). Natriuresis is determined by measuring the amount of sodium in the urine.		3896, 3897 3898 3899 3900	
BDNF isoform c (brain-derived neurotrophic factor)	Neurotrophic factor that promotes neuronal growth differentiation, and survival; maintains the survival of subsets of peripheral and central neurons during development plays a role in a adult nervous system function; may contribute to the nociceptive responses.	BDNF activity on neuronal growth can be measured using neuronal growth and synaptic activity assays, such as those described in Bartrup et al (1997) Neuroreport 1 8(17):3791 4, BDNF activity on pain reception can be assayed by measuring nociceptive behaviors hyperalgesia, and/or allodynia as described in Shu et al Pain (1999) 80:463-470 and in Zhou et al Eur J. Neurosci. (2000) 12 100-105.	Pain, Neuropathic pain, Complex regional pain syndrome I; Reflex sympathetic dystrophy Trigeminal neuralgia, Allodynia, Primary and/or Secondary hyperalgesia; Causalgia; Phantom limb pain, Post-surgical pain, Burning feet syndrome; Guillian-Barre syndrome; Thalamic syndrome; Post-stroke pain Vasculitic/ mapopathic pain, Idiopathic pain Pain associated with any of the following: Entrapment neuropathy Nerve transection, Spinal cord injury Scar formation, Alcoholic neuropathy Pellagra, Refsum, Post-herpetic neuralgia, HIV/AIDS pain, Vincristine neurotoxicity Cisplatin neurotoxicity Taxol neurotoxicity Thalium neurotoxicity Arsenic neurotoxicity, Radiation therapy, Diabetes, Malignancies, Multiple sclerosis, Fabry's disease, Tangier disease, or Amyloid.	3549	See Table 2 SEQ ID NO.Z for particular construct.
Glucagon-Like-Peptide 1 (GLP1 GLP 1 Insulinotropin)	Stimulates the synthesis and release of insulin enhances the sensitivity of adipose, muscle, and liver tissues towards insulin stimulates glucose uptake; slows the digestive process; suppresses appetite blocks the secretion of glucagon	GLP1 activity may be assayed in vitro using a [3-H]-glucose uptake assay (J Biol Chem 1999 Oct 22, 274(43).30864-30873) GLP-1 effects on learning can be investigated using the passive avoidance and Morris water maze (MWM) paradigms in rats (Brain Res. 1996, 716:29-38 and Nature 1982, 297:681-683).	Hyperglycemia, Diabetes, Diabetes Insipidus, Diabetes mellitus Type 1 diabetes; Type 2 diabetes; Insulin resistance; Insulin deficiency Hypertriglicemia, Hypercholesterolemia, Non-insulin dependent Diabetes Mellitus (NIDDM); Insulin-dependent Diabetes Mellitus (IDDM); A Condition Associated With Diabetes Including, But Not Limited To Obesity Heart Disease, Hyperglycemia, Infections, Retinopathy And/Or Ulcers; Metabolic Disorders, Immune Disorders, Obesity Vascular Disorders; Suppression of Body Weight; Suppression of Appetite; Syndrome X, Cognitive Impairment; Memory Loss Use to enhance learning, memory, associative learning, spatial learning, hippocampal plasticity cognition, and/or neuroprotection.	3610, 3696.	See Table 2, SEQ ID NO:Z for particular construct.
Lysosomal glucocerebrosidase (Alglucerase; β-glucocerebrosidase; β-D-glucosyl-*N*-acylsphingosine glucohydrolase; Glucosylceramidase; and β-	Catalyzes the hydrolysis of D-glucosyl-*N*-acylsphingosine to D-glucose and N acylsphingosine, catalyzes the hydrolysis of glucocerebroside to glucose ceramide	Enzymatic activity can be assessed using methods known in the art, for example, the thin-layer chromatography assay described by Poulos et al. Clin. Chim Acta. 1976 Nov 72(3):327-335 or the FACS method described by Rudensky et al., Blood Cells Mol. Dis. 2003	Gaucher disease, lysosomal storage diseases	3920 3921 3922 3923.	See Table 2 SEQ ID NO:Z for particular construct. Also, for particular lysosomal glucocerebrosidase sequences or variant

Table 1

Therapeutic Protein X	Biological Activity	Exemplary Activity Assay	Preferred Indication Y	Construct ID	Therapeutic Protein Z
glucosidase; imiglucerase CEREDASE, CEREZYME) (GCB)		Jan-Feb; 30(1):97-99.			thereof, see GLCM_HUMAN Genbank Accession No. P04062, CAS-143003-46-7 each of which is incorporated by reference. Other lysosomal glucocerebrosidase sequence are disclosed in U.S Patent Pub Nos. 20040009165, 20030133924, 20020127219, each of which is incorporated by reference. Additional lysosomal glucocerebrosidases are disclosed in U.S Patent Nos. 6,696,272 5,879,680-5,549,892, each of which is incorporated by reference
Arginine deiminase (ADI-SS)	Catalyzes the irreversible hydrolysis of arginine to citrulline and ammonia, inhibits nitric oxide synthesis inhibits angiogenesis; inhibits proliferation of numerous tumor cells.	Enzymatic activity can be measured using methods well known in the art, such as the enzyme assay described in Wesekmann et al., J Biol Chem 1977 Apr 252(8):2615-2620 Inhibition of angiogenesis can be assessed using known methods in the art, for example, the Matrigel assay as described in Park et al. Br J Cancer 2003 Sept; 89(5):907-914. Inhibition of nitric oxide production can be assessed using known methods in the art, for example, as described in Noh et al., Mol. Cells 2002 Feb:13(1):137 143 Tumor cell proliferation can be assessed using known methods in the art, for example, the proliferation assay as described in Miyazaki et al., Cancer Res. 1990 Aug; 50(15):4522-4527	Cancer including but not limited to melanoma and hepatocellular carcinoma. Additional cancers include, but are limited to, squamous cell carcinom osteosarcoma, glioma/astrocytoma, glioblastoma, promyelocytic leukemia, lymphoblastic leukemia, and carcinomas of the cervix, breast, ovaries, prostate, colon, or lung.	3910, 3915, 3917 3918.	See Table 2 SEQ ID NO:2 for particular construct. Also, for particular arginine deiminase sequence or variant thereof, see Baur et al., Eur J Biochem 1989 Jun, 179(1):53-60· Misawa et al., J Biotechnol. 1994 361 145 155 each of which is incorporated by reference. Other arginine deiminase sequences are disclosed in U.S. Patent Pub No. 20040096437 incorporated by reference. Other arginine deiminases are disclosed in, U.S Patent Nos. 6,180,387 5,804,183 5,474,928 5,196,195, each of which

Table 1

Therapeutic Protein:X	Biological Activity	Exemplary Activity Assay	Preferred Indication Y	Construct ID	Therapeutic Protein:Z
					is incorporated by reference.
Uricase (Urate oxidase; *Aspergillus flavus* uricase; *Candida utilis* uricase; rasburicase FASTURTEC; ELITEK)	Catalyzes the oxidation of uric acid into allantoin	Levels of uric acid can be measured spectrophotometrically, colorimetrically or using other methods that are well known in the art, such as in Kageyama, Clin. Chim Acta. 1971 31(2):421-426.	Gout; hyperuricemia; tumor lysis hyperuricea, chemotherapy-induced hyperuricea, ischemic stroke; lipid abnormalities cardiovascular disorders, including but not limited to hypertension, coronary artery disease, atherosclerosis.	3903 3904, 3936, 3937 3938, 3939 3940 3941 3942, 3943, 3944.	See Table 2, SEQ ID NO:Z for particular construct. Also, for particular uricase sequence or variant thereof see CAS-134774-45.1 Wu et al., P N.A.S. USA 1989 Dec 86(23):9412-9416, each of which is incorporated by reference. Other uricase sequences are disclosed in U.S Patent Nos. 4,062,731 4,273,874, 4,987,076, 5,376,545 5,700,674 5,728,562, 5,801,036; 5,834,273, 5,955,336; each of which is incorporated by reference
Interferon Hybrids specifically preferred: IFNalpha A/D hybrid (BglII version) IFNalpha A/D hybrid (PvuII version) IFNalpha A/F hybrid IFNalpha A/B hybrid IFNbeta 1/alpha D hybrid (IFNbeta-1/alpha-1 hybrid) IFNalpha/beta hybrid	Confers a range of cellular responses including antiviral, antiproliferative, antitumor and immunomodulatory activities; stimulate production of two enzymes a protein kinase and an oligoadenylate synthetase. Also, modulates MHC antigen expression NK cell activity and IFNg production and IL12 production in monocytes.	Anti-viral assay- Rubinstein S, Familletti PC, Pestka S (1981) Convenient assay for interferons. J Virol. 37(2):755-8, Anti-proliferation assay- Gao Y et al (1999) Sensitivity of an epstein-barr virus-positive tumor line, Daudi, to alpha interferon correlates with expression of a GC-rich viral transcript. Mol Cell Biol. 19(11):7305 13.	Viral infections include Severe Acute Respiratory Syndrome (SARS) and other coronavirus infections; filoviruses, including but not limited to Ebola viruses and Marburg virus Viral infections; HIV infections, Hepatitis; Arenaviruses, including but not limited to Pichande virus, Lassa virus, Junin virus, Machupo virus, Guanarito virus and lymphocytic choriomeningitis virus (LCMV) Bunyaviruses, including but not limited to Punta toro virus, Crimean-Congo hemorrhagic fever virus, sandfly fever viruses, Rift Valley fever virus, La Crosse virus, and hantaviruses; Flaviviruses including but not limited to Yellow Fever Banzi virus, West Nile virus, Dengue viruses, Japanese Encephalitis virus, Tick-borne encephalitis, Omsk Hemorrhagic Fever and Kyasanur Forest Disease virus; Togaviruses, including but not limited to Venezuelan, eastern, and western equine encephalitis viruses, Ross River virus, and Rubella virus Orthopox viruses, including but not limited to Vaccinia, Cowpox, Smallpox, and Monkeypox, Herpesviruses; FluA/B; Respiratory Syncytial virus (RSV), parafluenzae, measles, rhinoviruses, adenoviruses; Semliki Forest virus,	2872, 2873, 2874, 2875, 2876.	See Table 2, SEQ ID NO:Z for particular construct.

Table 1

Therapeutic Protein X	Biological Activity	Exemplary Activity Assay	Preferred Indication Y	Construct ID	Therapeutic Protein Z
			Viral Hemorrhagic fevers, Rhabdoviruses; Paramyxoviruses, including but not limited to Nipah virus and Hendra virus, and other viral agents identified by the U.S. Centers for Disease Control and Prevention as high-priority disease agents (i.e. Category A, B and C agents; see, e.g. Morse, Emerg. Med. Clin. North. Am. 2002 20(2):311-30 and Darling et al., Emerg. Med. Clin. North Am. 2002;20(2):273-309).		
IL-2 (Aldesleukin Interleukin-2 fusion toxin T cell growth factor PROLEUKIN IMMUNACE, CELSUK, ONCOLIPIN 2 MACROLIN)	Promotes the growth of B and T cells and augments NK cell and CTL cell killing activity	T cell proliferation assay Biological activity of recombinant human interleukin-2 produced in Escherichia coli Science 223 1412-1415 1984 natural killer (NK) cell and CTL cytotoxicity assay "Control of homeostasis of CD8+ memory T cells by opposing cytokines. Science 288 675-678 2000 CTLL-2 Proliferation Gillis et al (1978) J Immunol 120, 2027	Cancer Solid Tumors; Pancreatic Cancer Colon Cancer Liver Cancer T-lymphomas; Graft-versus-host disease (GVH); Autoimmune diseases, Autoimmune disorders, IL-2 receptor positive malignancies.	1757 1758, 1812 1813 1952, 1954.	See Table 2, SEQ ID NO:Z for particular construct.
Adrenomedullin (ADM)	Inhibits renal Na+-K+-ATPase; enhances synthesis of prostaglandin E2 that regulates contraction and relaxation of smooth muscle, as well as the dilation and constriction of blood vessels, inhibits aldosterone secretion causes diuresis and natriuresis. Involved in regulation of blood pressure and salt/water/electrolyte balance in body fluids, renoprotection.	Renal Na+-K+-ATPase activity can be measured using assays known in the art, such as in Ka et al., 1987 Endocrinology 120:2166-2173. Vasodilation can be measured using assays known in the art (Ashton et al. Pharmacology 2000 61(2):101 105 Prostaglandin E2 synthesis can be determined using assays known in the art, (Cheng et al. J Endocrinol. 2004 Aug,182(2):249-56). Aldosterone levels can be measured using methods known in the art, for example, in Yamato et al., Circ J 2003 May 67(5):384-90. Blood pressure can be measured with a sphygmomanometer or using other methods known in the art, such as in Reddy et al., Ultrasound Med Biol 2003 Mar 29(3):379-85 Natriuresis is determined by measuring the amount of sodium in the urine. Diuresis is determined by measuring the amount of urine secreted.	Treatment of Congestive Heart Failure Hypertension, Cardiovascular diseases; Acute renal failure; Acute tubular necrosis; Renal diseases; Renal Glomerular disease; Cardiac volume overload; cardiac decompensation, Left Ventricular Dysfunction; Dyspnea. Treatment for elevated aldosterone levels, which can lead to vasoconstriction, impaired cardiac output and/or hypertension, cardiac failure; myocardial reperfusion injury left ventricle remodeling.	3924, 3925, 3926, 3927	See Table 2, SEQ ID NO:Z for particular construct. Also see, Vesely Am J Physiol Renal Physiol 2003 285.F167 177 which is incorporated by reference
Long-acting natriuretic peptide (LANP proANP-(31-67),	Inhibits renal Na -K -ATPase; enhances synthesis of prostaglandin E2 that regulates contraction and relaxation of smooth muscle, as well as the	Renal Na+-K+-ATPase activity can be measured using assays known in the art, such as in Ku et al., 1987 Endocrinology 120:2166-2173. Vasodilation can be measured using assays known in the art (Ashton et al.	Treatment of Congestive Heart Failure Hypertension Cardiovascular disease, Cardiac volume overload, cardiac decompensation; Left Ventricular Dysfunction Dyspnea, Acute tubular necrosis; Acute renal failure; kidney tubule	3886, 3887	See Table 2, SEQ ID NO:Z for particular construct. Also see, Vesely Am J Physiol Renal Physiol 2003, 285.F167-177 which

Table 1

Therapeutic Protein:X	Biological Activity	Exemplary Activity Assay	Preferred Indication:Y	Construct ID	Therapeutic Protein:Z
	dilation and constriction of blood vessels inhibits plasma renin activity causes diuresis and natriuresis. Involved in regulation of blood pressure and salt/water/electrolyte balance in body fluids renoprotection	Pharmacology 2000; 61(2):101 105. Prostaglandin E2 synthesis can be determined using assays known in the art, (Cheng et al J Endocrinol. 2004 Aug;182(2):249-56). Blood pressure can be measured with a sphygmomanometer or using other methods known in the art, such as in Reddy et al., Ultrasound Med Biol 2003 Mar 29(3):379-85 Natriuresis is determined by measuring the amount of sodium in the urine. Diuresis is determined by measuring the amount of urine secreted.	regeneration Chronic Renal failure; Renal disease Renal Glomerular disease.		is incorporated by reference
Vessel Dualator (VDP proANP-(79-98)).	Inhibits renal Na^+-K^+-ATPase, enhances synthesis of prostaglandin E2 that regulates contraction and relaxation of smooth muscle, as well as the dilation and constriction of blood vessels; inhibits aldosterone secretion, causes natriuresis in patients with congestive heart failure; causes kaliuresis Involved in regulation of blood pressure and salt/water/electrolyte balance in body fluids, renoprotection.	Renal Na^+-K^+-ATPase activity can be measured using assays known in the art, such as in Ku et al. 1987 Endocrinology 120 2166-2173 Vasodilation can be measured using assays known in the art (Ashton et al. Pharmacology 2000; 61(2):101 105. Prostaglandin E2 synthesis can be determined using assays known in the art, (Cheng et al. J Endocrinol. 2004 Aug;182(2):249-56). Aldosterone levels can be measured using methods known in the art, for example, in Yasuno et al., Circ J 2003, May 67(5) 384-90. Blood pressure can be measured with a sphygmomanometer or using other methods known in the art, such as in Reddy et al., Ultrasound Med Biol 2003 Mar 29(3):379-85. Natriuresis is determined by measuring the amount of sodium in the urine. Kaliuresis is determined by measuring the amount of potassium in the urine.	Treatment of Congestive Heart Failure; Hypertension; Cardiovascular disease; Cardiac volume overload, cardiac decompensation; Left Ventricular Dysfunction Dyspnea Acute tubular necrosis Acute renal failure Renal disease Renal Glomerular disease. Treatment for elevated aldosterone levels, which can lead to vasoconstriction, impaired cardiac output and/or hypertension, cardiac failure; myocardial reperfusion injury left ventricle remodeling.	3888 3889	See Table 2 SEQ ID NO:Z for particular construct. Also see, Vesely Am J Physiol Renal Physiol 2003 285.F167 177 which is hereby incorporated by reference
Kaliuretic Peptide (KUP proANP-(99-126)).	Involved in regulation of blood pressure and salt/water/electrolyte balance in body fluids.	Blood pressure can be measured with a sphygmomanometer or using other methods known in the art, such as in Reddy et al., Ultrasound Med Biol 2003 Mar 29(3):379-85. Natriuresis is determined by measuring the amount of sodium in the urine. Diuresis is determined by measuring the amount of urine secreted	Treatment of Congestive Heart Failure; Hypertension, Cardiovascular disease; Acute tubular necrosis, Acute renal failure, Renal disease; Renal Glomerular disease.	3890, 3891	See Table 2, SEQ ID NO:Z for particular construct. Also see, Vesely Am J Physiol Renal Physiol 2003 285.F167 177 which is hereby incorporated by reference
C-type Natriuretic Peptide (CNP)	Promotes diuresis and natriuresis. Involved in regulation of blood pressure and salt/water/electrolyte	Natriuresis is determined by measuring the amount of sodium in the urine. Diuresis is determined by measuring the amount of urine secreted.	Treatment of Congestive Heart Failure; Hypertension, Cardiovascular disease; Acute tubular necrosis; Acute renal failure; Renal disease; Renal Glomerular disease.	3892, 3893	See Table 2 SEQ ID NO:Z for particular construct. Also see, Vesely Am J Physiol Renal Physiol

Table 1

Therapeutic Protein X	Biological Activity	Exemplary Activity Assay	Preferred Indication Y	Construct ID	Therapeutic Protein Z
	balance in body fluids.				2003 285:F167-177 which is hereby incorporated by reference
Dendroaspis natriuretic peptide (DNP)	Inhibits Na+-K+-ATPase; enhances synthesis of prostaglandin E2 that regulates contraction and relaxation of smooth muscle, as well as the dilation and constriction of blood vessels; inhibits aldosterone secretion causes diuresis and natriuresis. Involved in regulation of blood pressure and salt/water/electrolyte balance in body fluids renoprotection.	Renal Na+-K+-ATPase activity can be measured using assays known in the art, such as in Ku et al. 1987- Endocrinology 120:2166-2173 Vasodilation can be measured using assays known in the art (Ashton et al. Pharmacology 2000- 61(2):101-105. Prostaglandin E2 synthesis can be determined using assays known in the art, (Cheng et al J Endocrinol 2004 Aug;182(2):249-56). Aldosterone levels can be measured using methods known in the art, for example, in Yasuda et al., Circ J 2003 May- 67(5):384-90 Blood pressure can be measured with a sphygmomanometer or using other methods known in the art, such as in Reddy et al. Ultrasound Med Biol 2003 Mar- 29(3):379-85 Natriuresis is determined by measuring the amount of sodium in the urine. Diuresis is determined by measuring the amount of urine secreted.	Treatment of Congestive Heart Failure Hypertension Cardiovascular disease; Acute renal failure; Acute tubular necrosis; Renal disease Renal Glomerular disease; Cardiac volume overload cardiac decompensation Left Ventricular Dysfunction, Dyspnea. Treatment for elevated aldosterone levels, which can lead to vasoconstriction, impaired cardiac output and/or hypertension- cardiac failure; myocardial reperfusion injury; left ventricle remodeling.	3894, 3895	See Table 2 SEQ ID NO:Z for particular construct. Also see, Vesely Am J Physiol Renal Physiol 2003 285:F167-177 which is hereby incorporated by reference
TweakR (Fn 14, Fibroblast growth factor-inducible 14)	Stimulates apoptotic cell death, induces angiogenesis; promotes migration and proliferation of endothelial cells, when mutated causes susceptibility to Klippel Trenaunay syndrome.	Apoptotic cell death can be measured using assays known in the art, for example, in Nakayama et al. J Immunol 2003 Jan 1 170(1):341-8). Endothelial cell migration and proliferation can be determined using methods known in the art, for example, in Woods et al J Biol Chem. 2002 Jan 18;277(3):1924-7	Treatment of Klippel-Trenaunay syndrome; Cancer- Solid Tumors; Pancreatic Cancer- Colon Cancer- Liver Cancer- Brain Cancer- Gastric Cancer- Lung Cancer- Breast Cancer- Bone Cancer- Autoimmune diseases Autoimmune disorders, Lupus, Rheumatoid arthritis Multiple sclerosis; Angiogenesis disorders Cardiovascular disease.	3919, 3932.	SEQ ID NO:Z for particular construct.
Kiss-1 (Metastin, kisspeptin-54)	Suppresses tumor metastasis activates G protein-coupled receptor GPR54 to inhibit tumor progression; controls gonadotropin secretion	Activation of GPR54 can be determined using assays known in the art, for example, in Becker et al. Biochem Biophys Res Commun. 2005 Jan 21;326(3):677-86. Gonadotropin secretion can be determined by measuring the amount of gonadotropin hormones in the serum	Treatment of Cancer- Gastric Carcinomas- Breast Carcinomas Esophageal Squamous Cell Carcinomas Bladder Cancer- Solid Tumors, Thyroid Cancer- Pancreatic Cancer- Colon Cancer- Liver Cancer- Lung Cancer- Brain Cancer- Bone Cancer- Gonadotropin deficiency- Infertility; Hypopituitarism; Panhypopituitarism; Oligomenorrhea; Amenorrhea, loss of libido; hot flushes; Dyspareunia, impotence, Osteoporis, Hypogonadotropic hypogonadism, Kallmann syndrome; Secondary hypogonadism.	3928 3929	See Table 2, SEQ ID NO:Z for particular construct. Also see, Dhar et al., Int J Cancer 2004 Oct 10;111(6):868-72, Harms et al. Clin Exp Metastasis 2003;20(1):11-8, or Gottsch et al. Endocrinology. 2004 Sep;145(9):4073-7 which are hereby incorporated by reference
Ephrin B1	Regulates tumor growth and	Tumor growth , angiogenesis, migration and	Cancer; Solid Tumors; Pancreatic Cancer; Colon	3930; 3931,	SEQ ID NO:Z for

Table 1

Therapeutic Protein-X	Biological Activity	Exemplary Activity Assay	Preferred Indication-Y	Construct ID	Therapeutic Protein-Z
(EFNB1)	angiogenesis; regulates migration and invasion of cancer cells; enhances platelet aggregation and clot maturation, Stimulates T-cell proliferation, lymphokine production, and CTL activity Stimulates endothelial cell migration and proliferation required for normal cochlear function	Invasion can be measured using assays known in the art (Nakada et al., Cancer Res 2004 May 1;64(9):3179-85 or Noren et al. Proc Natl Acad Sci U S A 2004 Apr 13 101(15):5583-8). T-cell proliferation, lymphokine production, and CTL activity can be determined using assays known in the art, for example, in Yu et al. J Biol. Chem. 2004, 279(33) 35531-35539. Endothelial cell migration and proliferation can be determined using methods known in the art, for example, in Woods et al. J Biol Chem. 2002 Jan 18;277(3) 1924-7 Cochlear function can be determined using assays known in the art (Howard et al., Hear Res. 2003 Apr-178(1 2) 118-30).	Cancer- Liver Cancer- Brain Cancer- Colorectal Cancer- Gastric Cancer- Lung Cancer- Bone Cancer- Breast Cancer- Cardiovascular Disease; Hepatocellular carcinoma; T-lymphomas; Graft-versus-host disease (GVH); Autoimmune diseases, Autoimmune disorders, Hemophilia, von Willebrand's disease; Bleeding disorders, Bleeding disorders resulting from Connective tissue disorders Leukemia, Myeloma, Heart/lung bypass Kidney dialysis; or drug-induced, Angiogenesis disorders; Cochlear disorders, Hearing loss; Vertigo; Tinnitus, Aural fullness, Acute and/or chronic inner ear disorders.	3934 3935.	particular construct.
B7-H3	Enhances tumor immunity- Regulates T-Cell activation and immune responses	Tumor immunity can be measured by methods known in the art, for example, in Luo et al J Immunol. 2004 Nov 1 173(9) 5445-50 T-cell activation can be measured using assays known in the art (Chapoval et al. Nat Immunol. 2001 Mar;2(3):269-74).	Cancer- Solid Tumors; Pancreatic Cancer- Colon Cancer- Liver Cancer- Lung Cancer- Bone Cancer- Breast Cancer- Brain Cancer., Autoimmune disease, Autoimmune Disorders, Osteolytic bone diseases, Hypercalcemia of malignancy- Malignancy-Induced bone pain.	3933	SEQ ID NO:Z for particular construct.

Table 2

Fusion No.	Construct ID	Construct Name	Description	Expression Vector	SEQ ID NO Y	SEQ ID NO:X	SEQ ID NO Z	SEQ ID NO-A	SEQ ID NO.B	Leader Sequence
1	3422	pSAC35.APsp.HSA IFNa	Acid Phosphatase signal peptide followed by mature HSA and IFNa.	pSAC35	149	117	181	213	214	Acid phosphatase
2	3423	pSAC35 INVsp.HSA.IFNa	Invertase signal peptid followed by mature HSA and IFNa.	pSAC35	150	118	182	215	216	Invertase
3	3424	pSAC35 KTsp.HSA IFNa	Killer Toxin signal peptide followed by mature HSA and IFNa.	pSAC35	151	119	183	217	218	Killer toxin
4	2249	pSAC35 IFNa2-HSA also named: pSAC23.IFNa2-HSA	Mature IFNa2 fused upstream of mature HSA and downstream of HSA/lsm2 leader sequence.	pSAC35	152	120	184	219	220	HSA/lsm2
5	2343	pSAC35 INV-IFNA2.HSA	Mature interferon alpha2 fused upstream of mature HSA and downstream of invertase signal peptide.	pSAC35	153	121	185	221	222	invertase
6	2366	pSAC35 MAF-IFNa2.HSA	Mature IFNa2 fused upstream of mature HSA and downstream of yeast mating factor alpha leader sequence.	pSAC35	154	122	186	223	224	MFa-1
7	2381	pC4 HSA-IFNa2(C17-E181)	Amino acids C17 to E181 of IFNa2 (fragment shown as amino acids C1 to E165 of SEQ ID NO:618) fused downstream of HSA.	pC4	155	123	187	225	226	HSA

Table 2

Fusion No.	Construct ID	Construct Name	Description	Expression Vector	SEQ ID NO:Y	SEQ ID NO:X	SEQ ID NO:Z	SEQ ID NO:A	SEQ ID NO:B	Leader Sequence
8	2382	pC4:IFNa2-HSA	IFNa2 fused upstream of mature HSA.	pC4	156	124	188	227	228	Native IFNa2 leader
9	2410	pSAC35INV IFNa-HSA	Mature IFNa2 fused downstream of the invertase signal peptide and upstream of mature HSA	pSAC35	157	125	189	229	230	invertase
10	3165	pSAC35.HSA IFNa also named CID 3165, pSAC35 HSA INFa	HSA fused upstream of IFNa and downstream of the HSA/mz2 leader	pSAC35	158	126	190			HSA/mz2
11	1778	pSAC35 IFNbeta.M22-N187 HSA	Residues M22-N187 of full-length IFNb (shown as M1 to N166 of SEQ ID NO:463) fused upstream of mature HSA and downstream of HSA/mz2 leader sequence.	pSAC35	159	127	191	231	232	HSA/mz2
12	1779	pSAC35.HSA IFNbeta.M22-N187	Residues M22-N187 of full-length IFNb (shown as M1 to N166 of SEQ ID NO:464) fused downstream of HSA with HSA/mz2 leader sequence.	pSAC35	160	128	192			HSA/mz2
13	2011	pC4 IFNb-HSA	Full length IFNb fused upstream of mature HSA.	pC4	161	129	193	233	234	Native IFNb leader
14	2013	pC4 HSA-IFNb.M22-N187	Amino acids M22 to N187 of IFNb (fragment shown as amino acids M1 to N166 of SEQ ID NO:527) fused downstream of HSA.	pC4	162	130	194			HSA
15	2033	pEE12.IFNb-HSA also named pEE12.1 IFNbeta-HSA	Full length IFNb fused upstream of mature HSA	pEE12.1	163	131	195			Native IFNb leader
16	2054	pEE12 HSA-IFNb	Mature IFNb fused downstream of HSA.	pEE12.1	164	132	196			HSA
17	2492	pC4.IFNb(deltaM22).HSA	Mature full length INFbeta fused upstream of mature HSA. First residue of native, mature IFNbeta (M22) has been deleted.	pC4	165	133	197			Native IFNβ leader
18	2580	pC4.IFNb(deltaM22,C38S)-HSA	IFNb fused upstream of mature HSA The IFNb used in this fusion lacks the first residue of the mature form of IFNb, which corresponds to M22 of SEQ ID NO:1687 Also amino acid 38 of SEQ ID NO:1687 has been mutated from Cys to Ser	pC4	166	134	198			Native IFNβ
19	2795	pC4.HSA(A14)-IFNb.M22-N187	The mature form of IFNb is fused to the C-terminus of HSA, which contains an modified signal peptide, designed to improve processing and homogeneity	pC4	167	135	199			Modified HSA (A14)
20	2796	pC4.HSA(S14)-IFNb.M22-N187	The mature form of IFNb is fused to the C-terminus of HSA, which contains a modified signal peptide, designed to improve processing and homogeneity	pC4	168	136	200			Modified HSA (S14)
21	2797	pC4 HSA(G14)-IFNb.M22-N187	The mature form of IFNb is fused to the C-terminus of HSA, which contains an modified signal peptide.	pC4	169	137	201			Modified HSA (G14)
22	2872	pSAC35.HSA.IFNaA(C1-Q91)/D(L93-E166)	This construct contains a hybrid form of IFNaA and IFNaD fused downstream of mature HSA.	pSAC35	170	138	202	235	236	HSA/mz2
23	2873	pSAC35 HSA IFNaA(C1-Q91)/B(L93-E166)	This construct contains a hybrid form of IFNaA and IFNaB fused downstream of mature HSA.	pSAC35	171	139	203	237	238	HSA/mz2
24	2874	pSAC35.HSA.IFNaA(C1-Q91)/F(L93-E166)	This construct contains a hybrid form of IFNaA and IFNaF fused downstream of mature HSA	pSAC35	172	140	204	239	240	HSA/mz2

Table 1

Fusion No.	Construct ID	Construct Name	Description	Expression Vector	SEQ ID NO:Y	SEQ ID NO:X	SEQ ID NO:Z	SEQ ID NO:A	SEQ ID NO:B	Leader Sequence
25	2875	pSAC35:HSA.IFNaA(C1Q-62)/D(Q64-E166)	This construct contains a hybrid form of IFNaA and IFNaD fused downstream of mature HSA.	pSAC35	173	141	205	241	242	HSA/kex2
26	2876	pSAC35:HSA.IFNaA(C1-Q91)/D(L93-E166); R23K,A113V	This construct contains a hybrid form of IFNaA and IFNaD fused downstream of mature HSA.	pSAC35	174	142	206	243	244	HSA/kex2
27	1757	pSAC35:IL2.A21-T153.145C/S.HSA	Mature human IL-2 with a single amino acid mutation (C to S at position 145) cloned downstream of the HSA/KEX2 leader and upstream of mature HSA	pSAC35	175	143	207			HSA/kex2
28	1758	pSAC35:HSA.IL2.A21-T153.145C/S	Mature human IL-2 with a single amino acid mutation (C to S at position 145) cloned downstream of HSA with HSA/kex2 leader sequence.	pSAC35	176	144	208			HSA/kex2
29	1812	pSAC35:IL2.A21-T153.HSA	Amino acids A21 to T153 of IL-2 fused downstream of the HSA/kex2 leader and upstream of mature HSA.	pSAC35	177	145	209			HSA/kex2
30	1813	pSAC35:HSA.IL2.A21-T153	Amino acids A21 to T153 of IL-2 fused downstream of HSA with HSA/kex2 leader sequence.	pSAC35	178	146	210			HSA/kex2
31	1952	pcDNA3.1:IL2.HSA	Full length human IL-2, having a Cysteine to Serine mutation at amino acid 145, fused upstream of mature HSA.	pCDNA3.1	179	147	211			Native IL-2 leader
32	1954	pC4:IL2.HSA	Full length human IL-2, having a Cysteine to Serine mutation at amino acid 145, fused upstream of mature HSA.	pC4	180	148	212			Native IL-2 leader
33	3468	pSAC35:KTsp.HSA.GH	Killer toxin signal peptide followed by mature HSA followed by growth hormone.	pSAC35	313	245	381	449	450	Kill toxin PrePro
34	3469	pSAC35:INVsp.HSA.GH	Invertase signal peptide followed by mature HSA followed by growth hormone.	pSAC35	314	246	382	451	452	Invertase
35	3470	pSAC35:APsp.HSA.GH	Acid phosphatase signal peptide followed by mature HSA followed by growth hormone.	pSAC35	315	247	383	453	454	Acid Phosphatase
36	3475	pSAC35:G19sp.HSA.GH	Modified HSA/Kex2 signal sequence followed by mature HSA followed by growth hormone	pSAC35	316	248	384	455	456	Modified HSA/Kex2
37	3476	pSAC35:G19sp.HSA.IFNa	The Modified HSA/kex2 signal sequence followed by mature HSA followed by INF-alpha.	pSAC35	317	249	385	457	458	Modified HSA/Kex2
38	3549	pSAC35:HSA/kex2.BDNFc.HSA	The HSA/kex2 leader followed by mature BDNFc (splice variant 6) followed by mature HSA.	pSAC35	318	250	386	None	None	HSA/Kex2
39	3610	pSAC35:(HSA/KEX(R19Q))SP.GLP-1(7-36A8G)x2.HSA	Modified HSA/kex2 leader followed by yeast codon optimized glp-1 dimer (where the second dimer has been altered so that the wobble position of each codon is different from the first dimer) fused to the N terminus of HSA.	pSAC35	319	251	387	459	460	Modified HSA/Kex2
40	3690	pC4:MPIFSP.BNP/HSA	Myeloid progenitor inhibitory factor-1 (MPIF) signal sequence followed by BNP fused to the N-terminus of mature HSA.	pC4	320	252	388	461	462	MPIF-1
41	3691	pC4:SPCON.BNP/HSA	A consensus signal sequence followed by BNP fused to the N-terminus of mature HSA.	pC4	321	253	389	463	464	Consensus
42	3696	pEE12.1:GLP-1(7-36(A8G)x2.HSA	Myeloid progenitor inhibitory factor-1 (MPIF) signal sequence followed by yeast codon optimized glp-1 dimer (where the second dimer has been altered so that the wobble position of each codon is different from the first dimer) fused to the N-terminus of HSA.	pEE12.1	322	254	390	465	466	MPIF-1

Table 2

Fusion No.	Construct ID	Construct Name	Description	Expression Vector	SEQ ID NO:Y	SEQ ID NO:X	SEQ ID NO:Z	SEQ ID NO:A	SEQ ID NO:B	Leader Sequence
43	3715	pSAC35:BNP29/HSA.S65	A single copy of human BNP (amino acids 1-29) fused to the N-terminus of HSA (S65-L585), an HSA N-terminal truncation (delta 1-64). Thus is downstream of the HSA/Kex2 signal sequence.	pSAC35	323	255	391	467	468	HSA/Kex2
44	3723	pEE12.1 MPIFSP BNP/HSA	Myeloid progenitor inhibitory factor-1 (MPIF) signal sequence followed by BNP fused to the N-terminus of mature HSA.	pEE12.1	324	256	392	None	None	MPIF 1
45	3724	pEE12.1.SPCON.BNP/HSA	A consensus signal sequence followed by BNP fused to the N terminus of mature HSA	pEE12.1	325	257	393	None	None	Consensus
46	3725	pEE12.1:SPCON2.BNP/HSA	A consensus signal sequence followed by BNP fused to the N terminus of mature HSA.	pEE12.1	326	258	394	None	None	Consensus Signal Peptide #2
47	3736	pC4.SPCON2 BNP/HSA	A consensus signal sequence followed by BNP fused to the N terminus of mature HSA	pC4	327	259	395	469	470	Consensus Signal Peptide #2
48	3769	pC4.BNP(R13G)/HSA	Myeloid progenitor inhibitory factor-1 (MPIF) signal sequence followed by BNP mutant (R13G) fused to the N-terminus of mature HSA	pC4	328	260	396	471	472	MPIF-1
49	3778	pC4:SPCON.BNP29/HSA.S65	A single copy of human BNP (1-29) fused to the N-terminus of HSA (S65-L585), an HSA N-terminal truncation (delta 1-64). This is downstream of a consensus signal sequence.	pC4	329	261	397	473	474	Consensus
50	3783	pC4:SPCON.BNP(R13G)/HSA	Consensus signal sequence followed by BNP mutant (R13G) fused to the N-terminus of mature HSA.	pC4	330	262	398	475	476	Consensus
51	3795	pC4:SPCON BNP(K14G)/HSA	Consensus signal sequence followed by BNP mutant (K14G) fused to the N-terminus of mature HSA.	pC4	331	263	399	477	478	Consensus
52	3796	pSAC35.HSA/BNP	Followed by mature HSA fused to the N-terminus of BNP	pSAC35	332	264	400	479	480	HSA/Kex2
53	3809	pSAC35.BNP30(GGG)/HSA	HSA/Kex2 signal sequence followed by BNP (amino acids 1-30) fused via tripartite glycines to the N-terminus of mature HSA	pSAC35	333	265	401	481	482	HSA/Kex2
54	3886	pSAC35.HSA/KEX2.LANP.HSA	HSA/Kex2 signal sequence followed by LANP fused to the N-terminus of mature HSA. LANP corresponds to amino acids 26-55 of SeqID No:402 (hereby referred to as LANP).	pSAC35	334	266	402	None	None	HSA/Kex2
55	3887	pSAC35.HSA/KEX2 HSA.LANP	HSA/Kex2 signal sequence followed by mature HSA fused to the N-terminus of mature LANP	pSAC35	335	267	403	None	None	HSA/Kex2
56	3888	pSAC35:HSA/KEX2.VDP HSA	HSA/Kex2 signal sequence followed by VDP fused to the N terminus of mature HSA. VDP corresponds to amino acids 56-92 of SeqID No:404 (hereby referred to as VDP)	pSAC35	336	268	404	None	None	HSA/Kex2
57	3889	pSAC35.HSA/KEX2.HSA VDP	HSA/Kex2 signal sequence followed by mature HSA fused to the N-terminus of mature VDP	pSAC35	337	269	405	None	None	HSA/Kex2
58	3890	pSAC35.HSA/KEX2.KUP HSA	HSA/Kex2 signal sequence followed by KUP fused to the N-terminus of mature HSA. KUP corresponds to amino acids 104-123 of SeqID No:406 (hereby referred to as KUP).	pSAC35	338	270	406	None	None	HSA/Kex2
59	3891	pSAC35.HSA/KEX2.HSA.KUP	HSA/Kex2 signal sequence followed by mature HSA fused to the N-terminus of mature KUP	pSAC35	339	271	407	None	None	HSA/Kex2

Table 2

Fusion No.	Construct ID	Construct Name	Description	Expression Vector	SEQ ID NO:Y	SEQ ID NO:X	SEQ ID NO:Z	SEQ ID NO:A	SEQ ID NO:B	Leader Sequence
60	3892	pSAC35:HSA/KEX2.CNP.HSA	HSA/Kex2 signal sequence followed by CNP fused to the N-terminus of mature HSA. CNP corresponds to amino acids 105-123 of SeqID No:408 (hereby referred to as KUP).	pSAC35	340	272	408	None	None	HSA/Kex2.
61	3893	pSAC35:HSA/KEX2.HSA.CNP	HSA/Kex2 signal sequence followed by mature HSA fused to the N-terminus of mature CNP.	pSAC35	341	273	409	None	None	HSA/Kex2
62	3894	pSAC35:HSA/KEX2.DNP.HSA	HSA/Kex2 signal sequence followed by DNP fused to the N-terminus of mature HSA.	pSAC35	342	274	410	None	None	HSA/Kex2
63	3895	pSAC35:HSA/KEX2.HSA.DNP	HSA/Kex2 signal sequence followed by mature HSA fused to the N-terminus of mature DNP.	pSAC35	343	275	411	None	None	HSA/Kex2
64	3896	pSAC35:HSA/KEX(R19Q)-BNP30(GGG).HSA	Modified HSA/KEX-2 leader followed by BNP (amino acids 1-30) fused via tripartite glycine linker to the N-terminus of mature HSA.	pSAC35	344	276	412	None	None	Modified HSA/Kex2
65	3897	pEE12.SPCON-BNP(K14Q).HSA	A consensus signal sequence followed by mutant BNP (K14Q) fused to the N-terminus of mature HSA.	pEE12.1	345	277	413	None	None	Consensus
66	3898	pSAC35:HSA/Kex-2-BNP(K14Q).HSA	HSA/KEX-2 signal sequence followed by mutant BNP (K14Q) fused to the N-terminus of mature HSA.	pSAC35	346	278	414	None	None	HSA/Kex2
67	3899	pSAC35:HSA/Kex-2(R19Q)-BNP(K14Q).HSA	Modified HSA/KEX-2 signal sequence followed by mutant BNP (K14Q) fused to the N-terminus of mature HSA.	pSAC35	347	279	415	None	None	Modified HSA/Kex2
68	3900	pSAC35:HSA/Kex-2-HSA.BNP(K14Q)	HSA/KEX-2 signal sequence followed by mature HSA fused to the N-terminus of mutant BNP (K14Q).	pSAC35	348	280	416	None	None	HSA/Kex2
69	3903	pC4:SPCON-Uricase.HSA	A consensus signal sequence followed by uricase from rat fused to the N-terminus of mature HSA.	pC4	349	281	417	None	None	Consensus
70	3904	pC4:HSA/Kex2-HSA.Uricase	HSA/KEX-2 signal sequence followed by mature HSA fused to the N-terminus of uricase from rat.	pC4	350	282	418	None	None	HSA/Kex2
71	3910	pSAC35:HSA/Kex-2-ADI.HSA	HSA/KEX-2 signal sequence followed by arginine deiminase from mycoplasma fused to the N-terminus of mature HSA.	pSAC35	351	283	419	None	None	HSA/Kex2
72	3915	pC4:SPCON-ADI.HSA	A consensus signal sequence followed by arginine deiminase from mycoplasma fused to the N-terminus of mature HSA.	pC4	352	284	420	None	None	Consensus
73	3917	pC4:HSA/Kex-2-HSA.ADI	HSA/KEX-2 signal sequence followed by mature HSA fused to the N-terminus of arginine deiminase from mycoplasma.	pC4	353	285	421	None	None	HSA/Kex2
74	3918	pSAC35:HSA/Kex2-HSA.ADI	HSA/KEX-2 signal sequence followed by mature HSA fused to the N-terminus of arginine deiminase from mycoplasma.	pSAC35	354	286	422	None	None	HSA/Kex2
75	3919	pSAC35:KEX2.TWEAKR.E28-F75.HSA	HSA/KEX-2 signal sequence followed by TweakR (amino acids 28-75) fused to the N-terminus of mature HSA. TweakR E28-F75 corresponds to amino acids 28-75 of SeqID No:423 (hereby referred to as TweakR E28-F75).	pSAC35	355	287	423	None	None	HSA/Kex2
76	3920	pSAC35:HSA/Kex2-GCB.HSA	HSA/KEX-2 signal sequence followed by lysosomal glucocerebrosidase fused to the N-terminus of mature HSA.	pSAC35	356	288	424	None	None	HSA/Kex2
77	3921	pC4:SPCON-GCB.HSA	A consensus signal sequence followed by lysosomal glucocerebrosidase fused to the N-terminus of mature HSA.	pC4	357	289	425	None	None	Consensus
78	3922	pSAC35:HSA/Kex2-HSA.GCB	HSA/KEX-2 signal sequence followed by mature HSA fused to the N-terminus of lysosomal glucocerebrosidase.	pSAC35	358	290	426	None	None	HSA/Kex2

Table 2

Fusion No	Construct ID	Construct Name	Description	Expression Vector	SEQ ID NO:Y	SEQ ID NO:X	SEQ ID NO:Z	SEQ ID NO A	SEQ ID NO B	Leader Sequence
79	3923	pC4.HSA/Kex2-HSA.OCB	HSA/KEX-2 signal sequence followed by mature HSA fused to the N-terminus of lysosomal glucocerebrosidase.	pC4	359	291	427	None	None	HSA/Kex2
80	3924	pSAC35 HSA/Kex2-ADM HSA	HSA/KEX-2 signal sequence followed by adrenomedullin fused to the N-terminus of mature HSA.	pSAC35	360	292	428	None	None	HSA/Kex2
81	3925	pC4:SPCON-ADM HSA	A consensus signal sequence followed by adrenomedullin fused to the N-terminus of mature HSA.	pC4	361	293	429	None	None	Consensus
82	3926	pSAC35.HSA/Kex2-HSA.ADM	HSA/KEX-2 signal sequence followed by mature HSA fused to the N-terminus of adrenomedullin.	pSAC35	362	294	430	None	None	HSA/Kex2
83	3927	pC4.HSA/Kex2-HSA-ADM	HSA/KEX-2 signal sequence followed by mature HSA fused to the N-terminus of adrenomedullin	pC4	363	295	431	None	None	HSA/Kex2
84	3928	pc4 HSA.Kiss.E20-Q145	The Pre-pro-region of the HSA signal sequence followed by mature HSA fused to the N-terminus of Kiss (amino acids 20-145). Kiss E20-Q145 corresponds to amino acids 20-145 of SeqID No:432 (hereby referred to as Kiss E20-Q145).	pC4	364	296	432	None	None	HSA Pre-Pro
85	3929	pSAC35:KEX2.HSA KISS-1 E20-Q145	HSA/KEX-2 signal sequence followed by mature HSA fused to the N-terminus of Kiss (amino acids 20-145).	pSAC35	365	297	433	None	None	HSA/Kex2
86	3930	pSAC35:KEX2.EphrmB1.K30-D229 HSA	HSA/KEX-2 signal sequence followed by Ephrin B1 (amino acids 30-229) fused to the N-terminus of mature HSA Ephrin B1 K30-D229 corresponds to amino acids 30-229 of SeqID No:434 (hereby referred to as Ephrin B1 K30-D229).	pSAC35	366	298	434	None	None	HSA/Kex2
87	3931	pc4:EphrmB1.M1-D229.HSA	Ephrin B1 (amino acids 1-229), including the native Ephrin B1 signal sequence, fused to the N-terminus of mature HSA	pC4	367	299	435	None	None	Native Ephrin B1
88	3932	pc4:TWEAKR.M1-P75.HSA	TweakR (amino acids 1 75), including the native TweakR signal sequence, fused to the N-terminus of mature HSA. TweakR M1 P75 corresponds to amino acids 1 75 of SeqID No:436 (hereby referred to as TweakR M1-P75).	pC4	368	300	436	None	None	Native TweakR
89	3933	pc4 B7-H3.M1-E247 HSA	B7-H3 (amino acids 1-247), including the native B7-H3 signal sequence, fused to the N-terminus of mature HSA.	pC4	369	301	437	None	None	Native B7 H3
90	3934	pc4.HSA.EphrmB1.K30-D229	The pre-pro-region of the HSA signal sequence followed by mature HSA fused to the N-terminus of Ephrin B1 (amino acids 30-229).	pC4	370	302	438	None	None	HSA Pre-Pro
91	3935	pSAC35 HSA EphrnB1 K30-D229	HSA/Kex2 signal sequence followed by mature HSA fused to the N-terminus of Ephrin B1 (amino acids 30-229).	pSAC35	371	303	439	None	None	HSA/Kex2
92	3936	pc4.SPCON.cUricase.HSA	A consensus signal sequence followed by uricase from chimpanzee fused to the N-terminus of mature HSA.	pC4	372	304	440	None	None	Consensus
93	3937	pc4.HSA.cUricase	The pre-pro-region of the HSA signal sequence followed by mature HSA fused to the N-terminus of uricase from chimpanzee.	pC4	373	305	441	None	None	HSA Pre-Pro
94	3938	pc4:SPCON.bUricase.HSA	A consensus signal sequence followed by uricase fused to the N-terminus of mature HSA	pC4	374	306	442	None	None	Consensus
95	3939	pc4:HSA bUricase	The pre-pro-region of the HSA signal sequence followed by mature HSA fused to the N-terminus of uricase.	pC4	375	307	443	None	None	HSA Pre-Pro
96	3940	pc4:SPCON.bUricase.HSA	A consensus signal sequence followed by uricase from baboon fused to the N-terminus of mature HSA	pC4	376	308	444	None	None	Consensus

Table 2

Fusion No.	Construct ID	Construct Name	Description	Expression Vector	SEQ ID NO:Y	SEQ ID NO.X	SEQ ID NO:Z	SEQ ID NO A	SEQ ID NO:B	Leader Sequence
97	3941	pc4.HSA.bUricase	The pre-pro-region of the HSA signal sequence followed by mature HSA fused to the N-terminus of uricase from baboon.	pC4	377	309	445	None	None	HSA Pre-Pro
98	3942	pSAC35 KEX2.hUricase.HSA	HSA/Kex2 signal sequence followed by uricase fused to the N-terminus of mature HSA.	pSAC35	378	310	446	None	None	HSA/Kex2
99	3943	pSAC35 KEX2.cUricase.HSA	HSA/Kex2 signal sequence followed by uricase from chimpanzee fused to the N-terminus of mature HSA.	pSAC35	379	311	447	None	None	HSA/Kex2
100	3944	pSAC35 KEX2.bUricase HSA	HSA/Kex2 signal sequence followed by uricase from baboon fused to the N-terminus of mature HSA.	pSAC35	380	312	448	None	None	HSA/Kex2
101	3618	pC4:SPCON.BNP1-32(2x)HSA	A consensus signal sequence followed by two, tandem copies of mature BNP fused to the N-terminus of mature HSA.	pC4	483	484	485	463	464	Consensus

[0064] Table 2 provides a non-exhaustive list of polynucleotides of the invention comprising, or alternatively consisting of, nucleic acid molecules encoding an albumin fusion protein. The first column, "Fusion No." gives a fusion number to each polynucleotide. Column 2, "Construct ID" provides a unique numerical identifier for each polynucleotide of the invention. The Construct IDs may be used to identify polynucleotides which encode albumin fusion proteins comprising, or alternatively consisting of, a Therapeutic protein portion corresponding to a given Therapeutic Protein:X listed in the corresponding row of Table 1 wherein that Construct ID is listed in column 5. The "Construct Name" column (column 3) provides the name of a given albumin fusion construct or polynucleotide.

[0065] The fourth column in Table 2, "Description" provides a general description of a given albumin fusion construct, and the fifth column, "Expression Vector" lists the vector into which a polynucleotide comprising, or alternatively consisting of, a nucleic acid molecule encoding a given albumin fusion protein was cloned. Vectors are known in the art, and are available commercially or described elsewhere. For example, as described in the Examples, an "expression cassette" comprising, or alternatively consisting of, one or more of (1) a polynucleotide encoding a given albumin fusion protein, (2) a leader sequence, (3) a promoter region, and (4) a transcriptional terminator, may be assembled in a convenient cloning vector and subsequently be moved into an alternative vector, such as, for example, an expression vector including, for example, a yeast expression vector or a mammalian expression vector. In one embodiment, for expression in *S. cerevisiae*, an expression cassette comprising, or alternatively consisting of, a nucleic acid molecule encoding an albumin fusion protein is cloned into pSAC35. In another embodiment, for expression in CHO cells, an expression cassette comprising, or alternatively consisting of, a nucleic acid molecule encoding an albumin fusion protein is cloned into pC4. In a further embodiment, a polynucleotide comprising or alternatively consisting of a nucleic acid molecule encoding the Therapeutic protein portion of an albumin fusion protein is cloned into pC4:HSA. In a still further embodiment, for expression in NS0 cells, an expression cassette comprising, or alternatively consisting of, a nucleic acid molecule encoding an albumin fusion protein is cloned into pEE12. Other useful cloning and/or expression vectors will be known to the skilled artisan and are within the scope of the invention.

[0066] Column 6, "SEQ ID NO:Y," provides the full length amino acid sequence of the albumin fusion protein of the invention. In most instances, SEQ ID NO:Y shows the unprocessed form of the albumin fusion protein encoded – in other words, SEQ ID NO:Y shows the signal sequence, a HSA portion, and a therapeutic portion all encoded by the particular construct. Specifically contemplated by the present invention are all polynucleotides that encode SEQ ID NO:Y. When these polynucleotides are used to express the encoded protein from a cell, the cell's natural secretion and processing steps produces a protein that lacks the signal sequence listed in columns 4 and/or 11 of Table 2. The specific amino acid sequence of the listed signal sequence is shown later in the specification or is well known in the art. Thus, most preferred embodiments of the present invention include the albumin fusion protein produced by a cell (which would lack the leader sequence shown in columns 4 and/or 11 of Table 2). Also most preferred are polypeptides comprising SEQ ID NO:Y without the specific leader sequence listed in columns 4 and/or 11 of Table 2. Compositions comprising these two preferred embodiments, including pharmaceutical compositions, are also preferred. Moreover, it is well within the ability of the skilled artisan to replace the signal sequence listed in columns 4 and/or 11 of Table 2 with a different signal sequence, such as those described later in the specification to facilitate secretion of the processed albumin fusion protein.

[0067] The seventh column, "SEQ ID NO:X," provides the parent nucleic acid sequence from which a polynucleotide encoding a Therapeutic protein portion of a given albumin fusion protein may be derived. In one embodiment, the parent nucleic acid sequence from which a polynucleotide encoding a Therapeutic protein portion of an albumin fusion protein may be derived comprises the wild type gene sequence encoding a Therapeutic protein shown in Table 1. In an alternative embodiment, the parent nucleic acid sequence from which a polynucleotide encoding a Therapeutic protein portion of an albumin fusion protein may be derived comprises a variant or derivative of a wild type gene sequence encoding a Therapeutic protein shown in Table 1, such as, for example, a synthetic codon optimized variant of a wild type gene sequence encoding a Therapeutic protein.

[0068] The eighth column, "SEQ ID NO:Z," provides a predicted translation of the parent nucleic acid sequence (SEQ ID NO:X). This parent sequence can be a full length parent protein used to derive the particular construct, the mature portion of a parent protein, a variant or fragment of a wildtype protein, or an artificial sequence that can be used to create the described construct. One of skill in the art can use this amino acid sequence shown in SEQ ID NO:Z to determine which amino acid residues of an albumin fusion protein encoded by a given construct are provided by the therapeutic protein. Moreover, it is well within the ability of the skilled artisan to use the sequence shown as SEQ ID NO:Z to derive the construct described in the same row. For example, if SEQ ID NO:Z corresponds to a full length protein, but only a portion of that protein is used to generate the specific CID, it is within the skill of the art to rely on molecular biology techniques, such as PCR, to amplify the specific fragment and clone it into the appropriate vector.

[0069] Amplification primers provided in columns 9 and 10, "SEQ ID NO:A" and "SEQ ID NO:B" respectively, are exemplary primers used to generate a polynucleotide comprising or alternatively consisting of a nucleic acid molecule encoding the Therapeutic protein portion of a given albumin fusion protein. In one embodiment of the invention, oligonucleotide primers having the sequences shown in columns 9 and/or 10 (SEQ ID NOS:A and/or B) are used to PCR amplify a polynucleotide encoding the Therapeutic protein portion of an albumin fusion protein using a nucleic acid molecule comprising or alternatively consisting of the nucleotide sequence provided in column 7 (SEQ ID NO:X)of the corresponding row as the template DNA. PCR methods are well-established in the art. Additional useful primer sequences could readily be envisioned and utilized by

those of ordinary skill in the art.

[0070] In an alternative embodiment, oligonucleotide primers may be used in overlapping PCR reactions to generate mutations within a template DNA sequence. PCR methods are known in the art.

[0071] As shown in Table 3, certain albumin fusion constructs disclosed in this application have been deposited with the ATCC®.

Table 3

Construct ID	Construct Name	ATCC Deposit No./ Date
1812	pSAC35:IL2.A21-T153.HSA	PTA-3759 Oct. 4, 2001
2053	pEE12:IFNb-HSA also named pEE12.1:IFNβ-HSA	PTA-3764 Oct. 4, 2001
2054	pEE12:HSA-IFNb	PTA-3941 Dec. 19, 2001
2249	pSAC35:IFNa2-HSA also named pSAC23:IFNα2-HSA	PTA-3763 Oct. 4, 2001
2343	pSAC35.INV-IFNA2.HSA	PTA-3940 Dec. 19, 2001
2381	pC4:HSA-IFNa2(C17-E181)	PTA-3942 Dec. 19, 2001
2382	pC4:IFNa2-HSA	PTA-3939 Dec. 19, 2001
2492	pC4.IFNb(delnM22).HSA	PTA-3943 Dec. 19, 2001
3165	pSAC35:HSA.IFNa also named CID 3165, pSAC35:HSA.INFa	PTA-4670 Sept. 16, 2002

[0072] It is possible to retrieve a given albumin fusion construct from the deposit by techniques known in the art and described elsewhere herein (see, Example 10). The ATCC is located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC deposits were made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure.

[0073] In a further embodiment of the invention, an "expression cassette" comprising, or alternatively consisting of one or more of (1) a polynucleotide encoding a given albumin fusion protein, (2) a leader sequence, (3) a promoter region, and (4) a transcriptional terminator can be moved or "subcloned" from one vector into another. Fragments to be subcloned may be generated by methods well known in the art, such as, for example, PCR amplification (e.g., using oligonucleotide primers having the sequence shown in SEQ ID NO:A or B), and/or restriction enzyme digestion.

[0074] In preferred embodiments, the albumin fusion proteins of the invention are capable of a therapeutic activity and/or biologic activity corresponding to the therapeutic activity and/or biologic activity of the Therapeutic protein corresponding to the Therapeutic protein portion of the albumin fusion protein listed in the corresponding row of Table 1. In further preferred embodiments, the therapeutically active protein portions of the albumin fusion proteins of the invention are fragments or variants of the protein encoded by the sequence shown in SEQ ID NO:X column of Table 2, and are capable of the therapeutic activity and/or biologic activity of the corresponding Therapeutic protein.

Polypeptide and Polynucleotide Fragments and Variants

Fragments

[0075] The present invention is further directed to fragments of the Therapeutic proteins described in Table 1, albumin proteins, and/or albumin fusion proteins of the invention.

[0076] The present invention is also directed to polynucleotides encoding fragments of the Therapeutic proteins described in Table 1, albumin proteins, and/or albumin fusion proteins of the invention.

[0077] Even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the Therapeutic protein, albumin protein, and/or albumin fusion protein of the invention, other Therapeutic activities and/or functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) may still be retained. For example, the ability of polypeptides with N-terminal deletions to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptides generally will be retained when less than the majority of the residues of the complete polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mutein with a large number of deleted N-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[0078] Accordingly, fragments of a Therapeutic protein corresponding to a Therapeutic protein portion of an albumin fusion protein of the invention, include the full length protein as well as polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the reference polypeptide (i.e., a Therapeutic protein referred to in Table 1, or a Therapeutic protein portion of an albumin fusion

protein encoded by a polynucleotide or albumin fusion construct described in Table 2). In particular, N-terminal deletions may be described by the general formula m to q, where q is a whole integer representing the total number of amino acid residues in a reference polypeptide (e.g., a Therapeutic protein referred to in Table 1, or a Therapeutic protein portion of an albumin fusion protein of the invention, or a Therapeutic protein portion of an albumin fusion protein encoded by a polynucleotide or albumin fusion construct described in Table 2), and m is defined as any integer ranging from 2 to q minus 6. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0079] In addition, fragments of serum albumin polypeptides corresponding to an albumin protein portion of an albumin fusion protein of the invention, include the full length protein as well as polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the reference polypeptide (i.e., serum albumin, or a serum albumin portion of an albumin fusion protein encoded by a polynucleotide or albumin fusion construct described in Table 2). In preferred embodiments, N-terminal deletions may be described by the general formula m to 585, where 585 is a whole integer representing the total number of amino acid residues in mature human serum albumin (SEQ ID NO:1), and m is defined as any integer ranging from 2 to 579. Polynucleotides encoding these polypeptides are also encompassed by the invention. In additional embodiments, N-terminal deletions may be described by the general formula m to 609, where 609 is a whole integer representing the total number of amino acid residues in full length human serum albumin (SEQ ID NO:3), and m is defined as any integer ranging from 2 to 603. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0080] Moreover, fragments of albumin fusion proteins of the invention, include the full length albumin fusion protein as well as polypeptides having one or more residues deleted from the amino terminus of the albumin fusion protein (e.g., an albumin fusion protein encoded by a polynucleotide or albumin fusion construct described in Table 2; or an albumin fusion protein having the amino acid sequence disclosed in column 6 of Table 2). In particular, N-terminal deletions may be described by the general formula m to q, where q is a whole integer representing the total number of amino acid residues in the albumin fusion protein, and m is defined as any integer ranging from 2 to q minus 6. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0081] Also as mentioned above, even if deletion of one or more amino acids from the N-terminus or C-terminus of a reference polypeptide (e.g., a Therapeutic protein; serum albumin protein; or albumin fusion protein of the invention) results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) and/or Therapeutic activities may still be retained. For example the ability of polypeptides with C-terminal deletions to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking the N-terminal and/or C-terminal residues of a reference polypeptide retains Therapeutic activity can readily be determined by routine methods described herein and/or otherwise known in the art.

[0082] The present invention further provides polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of a Therapeutic protein corresponding to a Therapeutic protein portion of an albumin fusion protein of the invention (e.g., a Therapeutic protein referred to in Table 1, or a Therapeutic protein portion of an albumin fusion protein encoded by a polynucleotide or albumin fusion construct described in Table 2). In particular, C-terminal deletions may be described by the general formula 1 to n, where n is any whole integer ranging from 6 to q minus 1, and where q is a whole integer representing the total number of amino acid residues in a reference polypeptide (e.g., a Therapeutic protein referred to in Table 1, or a Therapeutic protein portion of an albumin fusion protein encoded by a polynucleotide or albumin fusion construct described in Table 2). Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0083] In addition, the present invention provides polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of an albumin protein corresponding to an albumin protein portion of an albumin fusion protein of the invention (e.g., serum albumin or an albumin protein portion of an albumin fusion protein encoded by a polynucleotide or albumin fusion construct described in Table 2). In particular, C-terminal deletions may be described by the general formula 1 to n, where n is any whole integer ranging from 6 to 584, where 584 is the whole integer representing the total number of amino acid residues in mature human serum albumin (SEQ ID NO:1) minus 1. Polynucleotides encoding these polypeptides are also encompassed by the invention. In particular, C-terminal deletions may be described by the general formula 1 to n, where n is any whole integer ranging from 6 to 608, where 608 is the whole integer representing the total number of amino acid residues in serum albumin (SEQ ID NO:3) minus 1. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0084] Moreover, the present invention provides polypeptides having one or more residues deleted from the carboxy terminus of an albumin fusion protein of the invention. In particular, C-terminal deletions may be described by the general formula 1 to n, where n is any whole integer ranging from 6 to q minus 1, and where q is a whole integer representing the total number of amino acid residues in an albumin fusion protein of the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0085] In addition, any of the above described N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted reference polypeptide. The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl terminal, which may be described generally as having residues m to n of a reference polypeptide (e.g., a Therapeutic protein referred to in Table 1, or a Therapeutic protein portion of an albumin fusion protein of the invention, or a Therapeutic protein portion encoded by a polynucleotide or albumin fusion

construct described in Table 2, or serum albumin (e.g., SEQ ID NO:1), or an albumin protein portion of an albumin fusion protein of the invention, or an albumin protein portion encoded by a polynucleotide or albumin fusion construct described in Table 2, or an albumin fusion protein, or an albumin fusion protein encoded by a polynucleotide or albumin fusion construct of the invention) where n and m are integers as described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0086] The present application is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a reference polypeptide sequence (e.g., a Therapeutic protein referred to in Table 1, or a Therapeutic protein portion of an albumin fusion protein of the invention, or a Therapeutic protein portion encoded by a polynucleotide or albumin fusion construct described in Table 2, or serum albumin (e.g., SEQ ID NO: 1), or an albumin protein portion of an albumin fusion protein of the invention, or an albumin protein portion encoded by a polynucleotide or albumin fusion construct described in Table 2, or an albumin fusion protein, or an albumin fusion protein encoded by a polynucleotide or albumin fusion construct of the invention) set forth herein, or fragments thereof. In preferred embodiments, the application is directed to proteins comprising polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to reference polypeptides having the amino acid sequence of N- and C-terminal deletions as described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0087] Preferred polypeptide fragments of the invention are fragments comprising, or alternatively, consisting of, an amino acid sequence that displays a Therapeutic activity and/or functional activity (e.g. biological activity) of the polypeptide sequence of the Therapeutic protein or serum albumin protein of which the amino acid sequence is a fragment.

[0088] Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Variants

[0089] "Variant" refers to a polynucleotide or nucleic acid differing from a reference nucleic acid or polypeptide, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the reference nucleic acid or polypeptide.

[0090] As used herein, "variant", refers to a Therapeutic protein portion of an albumin fusion protein of the invention, albumin portion of an albumin fusion protein of the invention, or albumin fusion protein of the invention differing in sequence from a Therapeutic protein (e.g. see "therapeutic" column of Table 1), albumin protein, and/or albumin fusion protein, respectively, but retaining at least one functional and/or therapeutic property thereof as described elsewhere herein or otherwise known in the art. Generally, variants are overall very similar, and, in many regions, identical to the amino acid sequence of the Therapeutic protein corresponding to a Therapeutic protein portion of an albumin fusion protein, albumin protein corresponding to an albumin protein portion of an albumin fusion protein, and/or albumin fusion protein. Nucleic acids encoding these variants are also encompassed by the invention.

[0091] The present invention is also directed to proteins which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%, identical to, for example, the amino acid sequence of a Therapeutic protein corresponding to a Therapeutic protein portion of an albumin fusion protein of the invention (e.g., the amino acid sequence of a Therapeutic protein:X disclosed in Table 1; or the amino acid sequence of a Therapeutic protein portion of an albumin fusion protein encoded by a polynucleotide or albumin fusion construct described in Table 1 and 2, or fragments or variants thereof), albumin proteins corresponding to an albumin protein portion of an albumin fusion protein of the invention (e.g., the amino acid sequence of an albumin protein portion of an albumin fusion protein encoded by a polynucleotide or albumin fusion construct described in Table 1 and 2; the amino acid sequence shown in SEQ ID NO: 1; or fragments or variants thereof), and/or albumin fusion proteins. Fragments of these polypeptides are also provided (e.g., those fragments described herein). Further polypeptides encompassed by the invention are polypeptides encoded by polynucleotides which hybridize to the complement of a nucleic acid molecule encoding an albumin fusion protein of the invention under stringent hybridization conditions (e.g., hybridization to filter bound DNA in 6X Sodium chloride/Sodium citrate (SSC) at about 45 degrees Celsius, followed by one or more washes in 0.2X SSC, 0.1% SDS at about 50 - 65 degrees Celsius), under highly stringent conditions (e.g., hybridization to filter bound DNA in 6X sodium chloride/Sodium citrate (SSC) at about 45 degrees Celsius, followed by one or more washes in 0.1X SSC, 0.2% SDS at about 68 degrees Celsius), or under other stringent hybridization conditions which are known to those of skill in the art (see, for example, Ausubel, F.M. et al., eds., 1989 *Current protocol in Molecular Biology*, Green publishing associates, Inc., and John Wiley & Sons Inc., New York, at pages 6.3.1 - 6.3.6 and 2.10.3). Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0092] By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, or substituted with another amino acid. These alterations of the reference sequence may occur at the amino- or carboxy-terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the

reference sequence or in one or more contiguous groups within the reference sequence.

[0093] As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence of an albumin fusion protein of the invention or a fragment thereof (such as a Therapeutic protein portion of the albumin fusion protein or an albumin portion of the albumin fusion protein), can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci.6:237-245 (1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is expressed as percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

[0094] If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C- terminal residues of the subject sequence.

[0095] For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

[0096] The variant will usually have at least 75 % (preferably at least about 80%, 90%, 95% or 99%) sequence identity with a length of normal HA or Therapeutic protein which is the same length as the variant. Homology or identity at the nucleotide or amino acid sequence level is determined by BLAST (Basic Local Alignment Search Tool) analysis using the algorithms employed by the programs blastp, blastn, blastx, tblastn and tblastx (Karlin *et al.*, Proc. Natl. Acad. Sci. USA 87: 2264-2268 (1990) and Altschul, J. Mol. Evol. 36: 290-300 (1993), fully incorporated by reference) which are tailored for sequence similarity searching.

[0097] The approach used by the BLAST program is to first consider similar segments between a query sequence and a database sequence, then to evaluate the statistical significance of all matches that are identified and finally to summarize only those matches which satisfy a preselected threshold of significance. For a discussion of basic issues in similarity searching of sequence databases, see Altschul *et al.*, (Nature Genetics 6: 119-129 (1994)) which is fully incorporated by reference. The search parameters for histogram, descriptions, alignments, expect (i.e., the statistical significance threshold for reporting matches against database sequences), cutoff, matrix and filter are at the default settings. The default scoring matrix used by blastp, blastx, tblastn, and tblastx is the BLOSUM62 matrix (Henikoff *et al.*, Proc. Natl. Acad. Sci. USA 89: 10915-10919 (1992), fully incorporated by reference). For blastn, the scoring matrix is set by the ratios of M (i.e., the reward score for a pair of matching residues) to N (i.e., the penalty score for mismatching residues), wherein the default values for M and N are 5 and -4, respectively. Four blastn parameters may be adjusted as follows: Q=10 (gap creation penalty); R=10 (gap extension penalty); wink=1 (generates word hits at every winkth position along the query); and gapw=16 (sets the window width within which gapped alignments are generated). The equivalent Blastp parameter settings were Q=9; R=2; wink=1; and gapw=32. A Bestfit comparison between sequences, available in the GCG package version 10.0, uses DNA parameters GAP=50 (gap creation penalty) and LEN=3 (gap extension penalty) and the equivalent settings in protein comparisons are GAP=8 and LEN=2.

[0098] The polynucleotide variants of the invention may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are

preferred. Moreover, polypeptide variants in which less than 50, less than 40, less than 30, less than 20, less than 10, or 5-50, 5-25, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host, such as, yeast or *E. coli*).

[0099] In a preferred embodiment, a polynucleotide of the invention which encodes the albumin portion of an albumin fusion protein is optimized for expression in yeast or mammalian cells. In a further preferred embodiment, a polynucleotide of the invention which encodes the Therapeutic protein portion of an albumin fusion protein is optimized for expression in yeast or mammalian cells. In a still further preferred embodiment, a polynucleotide encoding an albumin fusion protein of the invention is optimized for expression in yeast or mammalian cells.

[00100] In an alternative embodiment, a codon optimized polynucleotide which encodes a Therapeutic protein portion of an albumin fusion protein does not hybridize to the wild type polynucleotide encoding the Therapeutic protein under stringent hybridization conditions as described herein. In a further embodiment, a codon optimized polynucleotide which encodes an albumin portion of an albumin fusion protein does not hybridize to the wild type polynucleotide encoding the albumin protein under stringent hybridization conditions as described herein. In another embodiment, a codon optimized polynucleotide which encodes an albumin fusion protein does not hybridize to the wild type polynucleotide encoding the Therapeutic protein portion or the albumin protein portion under stringent hybridization conditions as described herein.

[0100] In an additional embodiment, a polynucleotide which encodes a Therapeutic protein portion of an albumin fusion protein does not comprise, or alternatively consist of, the naturally occurring sequence of that Therapeutic protein. In a further embodiment, a polynucleotide which encodes an albumin protein portion of an albumin fusion protein does not comprise, or alternatively consist of, the naturally occurring sequence of albumin protein. In an alternative embodiment, a polynucleotide which encodes an albumin fusion protein does not comprise, or alternatively consist of, the naturally occurring sequence of a Therapeutic protein portion or the albumin protein portion.

[0101] Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985)). These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

[0102] Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the polypeptide of the present invention without substantial loss of biological function. As an example, Ron et al. (J. Biol. Chem. 268: 2984-2988 (1993)) reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

[0103] Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem. 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

[0104] Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

[0105] Thus, the invention further includes polypeptide variants which have a functional activity (e.g., biological activity and/or therapeutic activity). In one embodiment, the invention provides variants of albumin fusion proteins that have a functional activity (e.g., biological activity and/or therapeutic activity) that corresponds to one or more biological and/or therapeutic activities of the Therapeutic protein corresponding to the Therapeutic protein portion of the albumin fusion protein. In another embodiment, the invention provides variants of albumin fusion proteins that have a functional activity (e.g., biological activity and/or therapeutic activity) that corresponds to one or more biological and/or therapeutic activities of the Therapeutic protein corresponding to the Therapeutic protein portion of the albumin fusion protein. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity. Polynucleotides encoding such variants are also encompassed by the invention.

[0106] In preferred embodiments, the variants of the invention have conservative substitutions. By "conservative substitutions" is intended swaps within groups such as replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys,

Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

[0107] Guidance concerning how to make phenotypically silent amino acid substitutions is provided, for example, in Bowie et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

[0108] The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

[0109] The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. See Cunningham and Wells, Science 244:1081-1085 (1989). The resulting mutant molecules can then be tested for biological activity.

[0110] As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly. Besides conservative amino acid substitution, variants of the present invention include (i) polypeptides containing substitutions of one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) polypeptides containing substitutions of one or more of the amino acid residues having a substituent group, or (iii) polypeptides which have been fused with or chemically conjugated to another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), (iv) polypeptide containing additional amino acids, such as, for example, an IgG Fc fusion region peptide. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

[0111] For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. See Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).

[0112] In specific embodiments, the polypeptides of the invention comprise, or alternatively, consist of, fragments or variants of the amino acid sequence of an albumin fusion protein, the amino acid sequence of a Therapeutic protein and/or human serum albumin, wherein the fragments or variants have 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, amino acid residue additions, substitutions, and/or deletions when compared to the reference amino acid sequence. In preferred embodiments, the amino acid substitutions are conservative. Nucleic acids encoding these polypeptides are also encompassed by the invention.

[0113] The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POST-TRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth. Enzymol.

182:626-646 (1990); Rattan et al., Ann. N.Y. Acad. Sci. 663:48-62 (1992)).

Functional activity

[0114] "A polypeptide having functional activity" refers to a polypeptide capable of displaying one or more known functional activities associated with the full-length, pro-protein, and/or mature form of a Therapeutic protein. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide for binding) to an anti-polypeptide antibody], immunogenicity (ability to generate antibody which binds to a specific polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide.

[0115] "A polypeptide having biological activity" refers to a polypeptide exhibiting activity similar to, but not necessarily identical to, an activity of a Therapeutic protein of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention).

[0116] In preferred embodiments, an albumin fusion protein of the invention has at least one biological and/or therapeutic activity associated with the Therapeutic protein portion (or fragment or variant thereof) when it is not fused to albumin.

[0117] In additional preferred embodiments, the albumin fusion protein of the invention has an increased plasma stability compared to the Therapeutic protein portion (or fragment or variant thereof) in an unfused state. Plasma stability of the albumin fusion protein of the invention or of the unfused Therapeutic protein portion (or fragment or variant thereof) can be assayed using or routinely modifying assays known in the art.

[0118] The albumin fusion proteins of the invention can be assayed for functional activity (e.g., biological activity) using or routinely modifying assays known in the art, as well as assays described herein. Additionally, one of skill in the art may routinely assay fragments of a Therapeutic protein corresponding to a Therapeutic protein portion of an albumin fusion protein, for activity using assays referenced in its corresponding row of Table 1 (e.g., in column 3 of Table 1). Further, one of skill in the art may routinely assay fragments of an albumin protein corresponding to an albumin protein portion of an albumin fusion protein, for activity using assays known in the art and/or as described in the Examples section below.

[0119] For example, in one embodiment where one is assaying for the ability of an albumin fusion protein to bind or compete with a Therapeutic protein for binding to an anti-Therapeutic polypeptide antibody and/or anti-albumin antibody, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

[0120] In a preferred embodiment, where a binding partner (e.g., a receptor or a ligand) of a Therapeutic protein is identified, binding to that binding partner by an albumin fusion protein which comprises that Therapeutic protein as the Therapeutic protein portion of the fusion can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky et al., Microbiol. Rev. 59:94-123 (1995). In another embodiment, the ability of physiological correlates of an albumin fusion protein to bind to a substrate(s) of the Therapeutic polypeptide corresponding to the Therapeutic protein portion of the fusion can be routinely assayed using techniques known in the art.

[0121] In an alternative embodiment, where the ability of an albumin fusion protein to multimerize is being evaluated, association with other components of the multimer can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky et al., *supra*.

[0122] In preferred embodiments, an albumin fusion protein comprising all or a portion of an antibody that binds a Therapeutic protein, has at least one biological and/or therapeutic activity (e.g., to specifically bind a polypeptide or epitope) associated with the antibody that binds a Therapeutic protein (or fragment or variant thereof) when it is not fused to albumin. In other preferred embodiments, the biological activity and/or therapeutic activity of an albumin fusion protein comprising all or a portion of an antibody that binds a Therapeutic protein is the inhibition (i.e., antagonism) or activation (i.e., agonism) of one or more of the biological activities and/or therapeutic activities associated with the polypeptide that is specifically bound by antibody that binds a Therapeutic protein.

[0123] Albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be characterized in a variety of ways. In particular, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be assayed for the ability to specifically bind to the same antigens specifically bound by the antibody that binds a Therapeutic protein

corresponding to the Therapeutic protein portion of the albumin fusion protein using techniques described herein or routinely modifying techniques known in the art.

[0124] Assays for the ability of the albumin fusion proteins (e.g., comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) to (specifically) bind a specific protein or epitope may be performed in solution (e.g., Houghten, Bio/Techniques 13:412-421(1992)), on beads (e.g., Lam, Nature 354:82-84 (1991)), on chips (e.g., Fodor, Nature 364:555-556 (1993)), on bacteria (e.g., U.S. Patent No. 5,223,409), on spores (e.g., Patent Nos. 5,571,698; 5,403,484; and 5,223,409), on plasmids (e.g., Cull et al., Proc. Natl. Acad. Sci. USA 89:1865-1869 (1992)) or on phage (e.g., Scott and Smith, Science 249:386-390 (1990); Devlin, Science 249:404-406 (1990); Cwirla et al., Proc. Natl. Acad. Sci. USA 87:6378-6382 (1990); and Felici, J. Mol. Biol. 222:301-310 (1991)) (each of these references is incorporated herein in its entirety by reference). Albumin fusion proteins comprising at least a fragment or variant of a Therapeutic antibody may also be assayed for their specificity and affinity for a specific protein or epitope using or routinely modifying techniques described herein or otherwise known in the art.

[0125] The albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be assayed for cross-reactivity with other antigens (e.g., molecules that have sequence/structure conservation with the molecule(s) specifically bound by the antibody that binds a Therapeutic protein (or fragment or variant thereof) corresponding to the Therapeutic protein portion of the albumin fusion protein of the invention) by any method known in the art.

[0126] Immunoassays which can be used to analyze (immunospecific) binding and cross-reactivity include, but are not limited to, competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

[0127] Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the albumin fusion protein of the invention (e.g., comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) to the cell lysate, incubating for a period of time (e.g., 1 to 4 hours) at 40 degrees C, adding sepharose beads coupled to an anti-albumin antibody, for example, to the cell lysate, incubating for about an hour or more at 40 degrees C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the albumin fusion protein to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the albumin fusion protein to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

[0128] Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), applying the albumin fusion protein of the invention (diluted in blocking buffer) to the membrane, washing the membrane in washing buffer, applying a secondary antibody (which recognizes the albumin fusion protein, e.g., an anti-human serum albumin antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., ^{32}P or ^{125}I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

[0129] ELISAs comprise preparing antigen, coating the well of a 96-well microtiter plate with the antigen, washing away antigen that did not bind the wells, adding the albumin fusion protein (e.g., comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) of the invention conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the wells and incubating for a period of time, washing away unbound or non-specifically bound albumin fusion proteins, and detecting the presence of the albumin fusion proteins specifically bound to the antigen coating the well. In ELISAs the albumin fusion protein does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes albumin fusion protein) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the albumin fusion protein may be coated to the well. In this case, the detectable molecule could be the antigen conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase). One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in

Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

[0130] The binding affinity of an albumin fusion protein to a protein, antigen, or epitope and the off-rate of an albumin fusion protein-protein/antigen/epitope interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (*e.g.*, ^{3}H or ^{125}I) with the albumin fusion protein of the invention in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the albumin fusion protein for a specific protein, antigen, or epitope and the binding off-rates can be determined from the data by Scatchard plot analysis. Competition with a second protein that binds the same protein, antigen or epitope as the albumin fusion protein, can also be determined using radioimmunoassays. In this case, the protein, antigen or epitope is incubated with an albumin fusion protein conjugated to a labeled compound (*e.g.*, ^{3}H or ^{125}I) in the presence of increasing amounts of an unlabeled second protein that binds the same protein, antigen, or epitope as the albumin fusion protein of the invention.

[0131] In a preferred embodiment, BIAcore kinetic analysis is used to determine the binding on and off rates of albumin fusion proteins of the invention to a protein, antigen or epitope. BIAcore kinetic analysis comprises analyzing the binding and dissociation of albumin fusion proteins, or specific polypeptides, antigens or epitopes from chips with immobilized specific polypeptides, antigens or epitopes or albumin fusion proteins, respectively, on their surface.

[0132] Antibodies that bind a Therapeutic protein corresponding to the Therapeutic protein portion of an albumin fusion protein may also be described or specified in terms of their binding affinity for a given protein or antigen, preferably the antigen which they specifically bind. Preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M. More preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6}M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M or 10^{-8} M. Even more preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M. In preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, has an affinity for a given protein or epitope similar to that of the corresponding antibody (not fused to albumin) that binds a Therapeutic protein, taking into account the valency of the albumin fusion protein (comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) and the valency of the corresponding antibody. In addition, assays described herein (see Examples and Table 1) and otherwise known in the art may routinely be applied to measure the ability of albumin fusion proteins and fragments, variants and derivatives thereof to elicit biological activity and/or Therapeutic activity (either *in vitro* or *in vivo*) related to either the Therapeutic protein portion and/or albumin portion of the albumin fusion protein. Other methods will be known to the skilled artisan and are within the scope of the invention.

Albumin

[0133] As described above, an albumin fusion protein of the invention comprises at least a fragment or variant of a Therapeutic protein and at least a fragment or variant of human serum albumin, which are associated with one another, preferably by genetic fusion.

[0134] An additional embodiment comprises at least a fragment or variant of a Therapeutic protein and at least a fragment or variant of human serum albumin, which are linked to one another by chemical conjugation.

[0135] The terms, human serum albumin (HSA) and human albumin (HA) are used interchangeably herein. The terms, "albumin and "serum albumin" are broader, and encompass human serum albumin (and fragments and variants thereof) as well as albumin from other species (and fragments and variants thereof).

[0136] As used herein, "albumin" refers collectively to albumin protein or amino acid sequence, or an albumin fragment or variant, having one or more functional activities (e.g., biological activities) of albumin. In particular, "albumin" refers to human albumin or fragments thereof (see for example, EP 201 239, EP 322 094 WO 97/24445, WO95/23857) especially the mature form of human albumin as shown in Figure 1 and SEQ ID NO: 1, or albumin from other vertebrates or fragments thereof, or analogs or variants of these molecules or fragments thereof.

[0137] In preferred embodiments, the human serum albumin protein used in the albumin fusion proteins of the invention contains one or both of the following sets of point mutations with reference to SEQ ID NO: 1: Leu-407 to Ala, Leu-408 to Val, Val-409 to Ala, and Arg-410 to Ala; or Arg-410 to A, Lys-413 to Gln, and Lys-414 to Gln (see, e.g., International Publication No. WO95/23857, hereby incorporated in its entirety by reference herein). In even more preferred embodiments, albumin fusion proteins of the invention that contain one or both of above-described sets of point mutations have improved stability/resistance to yeast YapJp proteolytic cleavage, allowing increased production of recombinant albumin fusion proteins expressed in yeast host cells.

[0138] As used herein, a portion of albumin sufficient to prolong the therapeutic activity or plasma stability or shelf-life of the Therapeutic protein refers to a portion of albumin sufficient in length or structure to stabilize or prolong the therapeutic activity or plasma stability of the protein so that the shelf life or plasma stability of the Therapeutic protein portion of the albumin fusion protein is prolonged or extended compared to the shelf-life or plasma stability in the non-fusion state. The albumin portion of the albumin fusion proteins may comprise the full length of the HA sequence as described above, or may include one or more fragments thereof that are capable of stabilizing or prolonging the therapeutic activity. Such fragments may be of 10 or more amino acids in length or may include about 15, 20, 25, 30, 50, or more contiguous amino acids from the HA

sequence or may include part or all of specific domains of HA. For instance, one or more fragments of HA spanning the first two immunoglobulin-like domains may be used. In a preferred embodiment, the HA fragment is the mature form of HA.

[0139] The albumin portion of the albumin fusion proteins of the invention may be a variant of normal HA. The Therapeutic protein portion of the albumin fusion proteins of the invention may also be variants of the Therapeutic proteins as described herein. The term "variants" includes insertions, deletions and substitutions, either conservative or non conservative, where such changes do not substantially alter one or more of the oncotic, useful ligand-binding and non-immunogenic properties of albumin, or the active site, or active domain which confers the therapeutic activities of the Therapeutic proteins.

[0140] In particular, the albumin fusion proteins of the invention may include naturally occurring polymorphic variants of human albumin and fragments of human albumin, for example those fragments disclosed in EP 322 094 (namely HA (Pn), where n is 369 to 419). The albumin may be derived from any vertebrate, especially any mammal, for example human, cow, sheep, or pig. Non-mammalian albumins include, but are not limited to, hen and salmon. The albumin portion of the albumin fusion protein may be from a different animal than the Therapeutic protein portion.

[0141] Generally speaking, an HA fragment or variant will be at least 100 amino acids long, preferably at least 150 amino acids long. The HA variant may consist of or alternatively comprise at least one whole domain of HA, for example domains 1 (amino acids 1-194 of SEQ ID NO: 1), domain 2 (amino acids 195-387 of SEQ ID NO:1), domain 3 (amino acids 388-585 of SEQ ID NO:1), domains 1 and 2 (1-387 of SEQ ID NO:1), domains 2 and 3 (195-585 of SEQ ID NO:1) or domains 1 and 3 (amino acids 1-194 of SEQ ID NO:1 and amino acids 388-585 of SEQ ID NO:1). Each domain is itself made up of two homologous subdomains namely 1-105, 120-194, 195-291, 316-387, 388-491 and 512-585, with flexible inter-subdomain linker regions comprising residues Lys106 to Glu119, Glu292 to Val315 and Glu492 to Ala511.

[0142] Preferably, the albumin portion of an albumin fusion protein of the invention comprises at least one subdomain or domain of HA or conservative modifications thereof. If the fusion is based on subdomains, some or all of the adjacent linker is preferably used to link to the Therapeutic protein moiety.

Antibodies that Specifically bind Therapeutic proteins are also Therapeutic proteins

[0143] The present invention also encompasses albumin fusion proteins that comprise at least a fragment or variant of an antibody that specifically binds a Therapeutic protein disclosed in Table 1. It is specifically contemplated that the term "Therapeutic protein" encompasses antibodies that bind a Therapeutic protein (e.g., as Described in column 1 of Table 1) and fragments and variants thereof. Thus an albumin fusion protein of the invention may contain at least a fragment or variant of a Therapeutic protein, and/or at least a fragment or variant of an antibody that binds a Therapeutic protein.

Antibody structure and background

[0144] The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. *See generally, Fundamental Immunology* Chapters 3-5 (Paul, W., ed., 4th ed. Raven Press, N.Y. (1998)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site.

[0145] Thus, an intact IgG antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same.

[0146] The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDR regions, in general, are the portions of the antibody which make contact with the antigen and determine its specificity. The CDRs from the heavy and the light chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains variable regions comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The variable regions are connected to the heavy or light chain constant region. The assignment of amino acids to each domain is in accordance with the definitions of Kabat *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk J *Mol. Biol.* 196:901-917 (1987); Chothia et al. *Nature* 342:878-883 (1989).

[0147] As used herein, "antibody" refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that specifically binds an antigen (e.g., a molecule containing one or more CDR regions of an antibody). Antibodies that may correspond to a Therapeutic protein portion of an albumin fusion protein include, but are not limited to, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies (e.g., single chain Fvs), Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies specific to antibodies of the invention), and epitope-binding fragments of any of the above (e.g., VH domains, VL domains, or one or more CDR regions).

Antibodies that bind Therapeutic Proteins

[0148] The present invention encompasses albumin fusion proteins that comprise at least a fragment or variant of an antibody that binds a Therapeutic Protein (e.g., as disclosed in Table 1) or fragment or variant thereof.

[0149] Antibodies that bind a Therapeutic protein (or fragment or variant thereof) may be from any animal origin, including birds and mammals. Preferably, the antibodies are human, murine (*e.g.*, mouse and rat), donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken antibodies. Most preferably, the antibodies are human antibodies. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries and xenomice or other organisms that have been genetically engineered to produce human antibodies.

[0150] The antibody molecules that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In preferred embodiments, the antibody molecules that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein are IgG1. In other preferred embodiments, the immunoglobulin molecules that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein are IgG2. In other preferred embodiments, the immunoglobulin molecules that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein are IgG4.

[0151] Most preferably the antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains.

[0152] The antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a Therapeutic protein or may be specific for both a Therapeutic protein as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

[0153] Antibodies that bind a Therapeutic protein (or fragment or variant thereof) may be bispecific or bifunctional which means that the antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. *See, e.g.*, Songsivilai & Lachmann *Clin. Exp. Immunol.* 79: 315-321 (1990), Kostelny et al. J *Immunol.* 148:1547 1553 (1992). In addition, bispecific antibodies may be formed as "diabodies" (Holliger et al. "Diabodies': small bivalent and bispecific antibody fragments" PNAS USA 90:6444-6448 (1993)) or "Janusins" (Traunecker et al. "Bispecific single chain molecules (Janusins) target cytotoxic lymphocytes on HIV infected cells" *EMBO J* 10:3655-3659 (1991) and Traunecker et al. "Janusin: new molecular design for bispecific reagents" *Int J Cancer Suppl* 7:51-52 (1992)).

[0154] The present invention also provides albumin fusion proteins that comprise, fragments or variants (including derivatives) of an antibody described herein or known elsewhere in the art. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding a molecule of the invention, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis which result in amino acid substitutions. Preferably, the variants (including derivatives) encode less than 50 amino acid substitutions, less than 40 amino acid substitutions, less than 30 amino acid substitutions, less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the reference VH domain, VHCDR1, VHCDR2, VHCDR3, VL domain, VLCDR1, VLCDR2, or VLCDR3. In specific embodiments, the variants encode substitutions of VHCDR3. In a preferred embodiment, the variants have conservative amino acid substitutions at one or more predicted non-essential amino acid residues.

[0155] Antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein may be described or specified in terms of the epitope(s) or portion(s) of a Therapeutic protein which they recognize or specifically bind. Antibodies which specifically bind a Therapeutic protein or a specific epitope of a Therapeutic protein may also be excluded. Therefore, the present invention encompasses antibodies that specifically bind Therapeutic proteins, and allows for the exclusion of the same. In preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, binds the same epitopes as the unfused fragment or variant of that antibody itself.

[0156] Antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a Therapeutic protein are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% sequence identity (as calculated using methods known in the art and described herein) to a Therapeutic protein are also included in the present invention. In specific embodiments, antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less

than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% sequence identity (as calculated using methods known in the art and described herein) to a Therapeutic protein are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. In preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, has similar or substantially identical cross reactivity characteristics compared to the fragment or variant of that particular antibody itself.

[0157] Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide encoding a Therapeutic protein under stringent hybridization conditions (as described herein). Antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M. More preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6}M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M or 10^{-8} M. Even more preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M. In preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, has an affinity for a given protein or epitope similar to that of the corresponding antibody (not fused to albumin) that binds a Therapeutic protein, taking into account the valency of the albumin fusion protein (comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) and the valency of the corresponding antibody.

[0158] The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of a Therapeutic protein as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%. In preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, competitively inhibits binding of a second antibody to an epitope of a Therapeutic protein. In other preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, competitively inhibits binding of a second antibody to an epitope of a Therapeutic protein by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

[0159] Antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention may act as agonists or antagonists of the Therapeutic protein. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described *supra*). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody. In preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, has similar or substantially similar characteristics with regard to preventing ligand binding and/or preventing receptor activation compared to an un-fused fragment or variant of the antibody that binds the Therapeutic protein.

[0160] The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the Therapeutic proteins (e.g. as disclosed in Table 1). The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties). In preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, have similar or substantially identical agonist or antagonist properties as an un-fused fragment or variant of the antibody that binds the Therapeutic protein.

[0161] Antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention may be used, for example, to purify, detect, and target Therapeutic proteins, including both in *in vitro* and *in vivo* diagnostic and therapeutic methods. For example, the antibodies have utility in immunoassays for qualitatively and quantitatively measuring levels of the Therapeutic protein in biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); incorporated by reference herein in its entirety. Likewise, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, may be used, for example, to purify, detect, and target Therapeutic proteins, including both *in vitro* and *in vivo* diagnostic and therapeutic methods.

[0162] Antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids. Albumin fusion proteins of the invention may also be modified as described above.

Methods of Producing Antibodies that bind Therapeutic Proteins

[0163] The antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a Therapeutic protein may be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

[0164] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., In: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

[0165] Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. In a non-limiting example, mice can be immunized with a Therapeutic protein or fragment or variant thereof, an albumin fusion protein, or a cell expressing such a Therapeutic protein or fragment or variant thereof or albumin fusion protein. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

[0166] Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

[0167] Another well known method for producing both polyclonal and monoclonal human B cell lines is transformation using Epstein Barr Virus (EBV). Protocols for generating EBV-transformed B cell lines are commonly known in the art, such as, for example, the protocol outlined in Chapter 7.22 of Current Protocols in Immunology, Coligan et al., Eds., 1994, John Wiley & Sons, NY, which is hereby incorporated in its entirety by reference. The source of B cells for transformation is commonly human peripheral blood, but B cells for transformation may also be derived from other sources including, but not limited to, lymph nodes, tonsil, spleen, tumor tissue, and infected tissues. Tissues are generally made into single cell suspensions prior to EBV transformation. Additionally, steps may be taken to either physically remove or inactivate T cells (e.g., by treatment with cyclosporin A) in B cell-containing samples, because T cells from individuals seropositive for anti-EBV antibodies can suppress B cell immortalization by EBV.

[0168] In general, the sample containing human B cells is innoculated with EBV, and cultured for 3-4 weeks. A typical source of EBV is the culture supernatant of the B95-8 cell line (ATCC #VR-1492). Physical signs of EBV transformation can generally be seen towards the end of the 3-4 week culture period. By phase-contrast microscopy, transformed cells may appear large, clear, hairy and tend to aggregate in tight clusters of cells. Initially, EBV lines are generally polyclonal. However, over prolonged periods of cell cultures, EBV lines may become monoclonal or polyclonal as a result of the selective outgrowth of particular B cell clones. Alternatively, polyclonal EBV transformed lines may be subcloned (e.g., by limiting dilution culture) or fused with a suitable fusion partner and plated at limiting dilution to obtain monoclonal B cell lines. Suitable fusion partners for EBV transformed cell lines include mouse myeloma cell lines (e.g., SP2/0, X63-Ag8.653), heteromyeloma cell lines (human x mouse; e.g, SPAM-8, SBC-H20, and CB-F7), and human cell lines (e.g., GM 1500, SKO-007, RPMI 8226, and KR-4). Thus, the present invention also provides a method of generating polyclonal or monoclonal human antibodies against polypeptides of the invention or fragments thereof, comprising EBV-transformation of human B cells.

[0169] Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')2 fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). F(ab')2 fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

[0170] For example, antibodies that bind to a Therapeutic protein can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make antibodies that bind to a Therapeutic protein include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

[0171] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

[0172] Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999 (1993); and Skerra et al., Science 240:1038-1040 (1988). For some uses, including *in vivo* use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

[0173] Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

[0174] Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced *randomly or by* homologous recombination *into mouse embryonic stem cells*. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, Int. Rev. Immunol. 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; 5,939,598; 6,075,181; and 6,114,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

[0175] Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., Bio/technology 12:899-903 (1988)).

Polynucleotides Encoding Antibodies

[0176] The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or alternatively, under lower stringency hybridization conditions, e.g., as defined *supra*, to polynucleotides that encode an antibody, preferably, that specifically binds to a Therapeutic protein, and more preferably, an *antibody that binds to a polypeptide having the amino acid sequence of a "Therapeutic protein:X" as disclosed in the "SEQ ID NO:Z" column of* Table 2.

[0177] The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0178] Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art (See Example 65).

[0179] Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

[0180] In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well know in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described *supra*. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed *supra*, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

[0181] In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described *supra*, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

[0182] Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423- 42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., Science 242:1038- 1041 (1988)).

Recombinant Expression of Antibodies

[0183] Recombinant expression of an antibody, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody or a single chain antibody), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

[0184] The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

[0185] A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., E. coli, B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., Saccharomyces, Pichia) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as Escherichia coli, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For

example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., Gene 45:101 (1986); Cockett et al., Bio/Technology 8:2 (1990)).

[0186] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the E. coli expression vector pUR278 (Ruther et al., EMBO J. 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, Nucleic Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem. 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[0187] In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

[0188] In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or *in vivo* recombination. Insertion in a non- essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

[0189] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

[0190] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

[0191] A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215 (1993)); and hygro, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be

routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

[0192] The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

[0193] Vectors which use glutamine synthase (GS) or DHFR as the selectable markers can be amplified in the presence of the drugs methionine sulphoximine or methotrexate, respectively. An advantage of glutamine synthase based vectors are the availability of cell lines (e.g., the murine myeloma cell line, NS0) which are glutamine synthase negative. Glutamine synthase expression systems can also function in glutamine synthase expressing cells (e.g. Chinese Hamster Ovary (CHO) cells) by providing additional inhibitor to prevent the functioning of the endogenous gene. A glutamine synthase expression system and components thereof are detailed in PCT publications: WO87/04462; WO86/05807; WO89/01036; WO89/10404; and WO91/06657 which are incorporated in their entireties by reference herein. Additionally, glutamine synthase expression vectors that may be used according to the present invention are commercially available from suppliers, including, for example Lonza Biologics, Inc. (Portsmouth, NH). Expression and production of monoclonal antibodies using a GS expression system in murine myeloma cells is described in Bebbington *et al.*, *Biotechnology* 10:169(1992) and in Biblia and Robinson *Biotechnol. Prog.* 11:1 (1995) which are incorporated in their entireties by reference herein.

[0194] The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

[0195] Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

Modifications of Antibodies

[0196] Antibodies that bind a Therapeutic protein or fragments or variants can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin tag (also called the "HA tag"), which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

[0197] The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include 125I, 131I, 111In or 99Tc. Other examples of detectable substances have been described elsewhere herein.

[0198] Further, an antibody or the invention may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, 213Bi. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thiotepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis- dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

[0199] The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, B-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF-alpha, TNF-beta, AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al., Int. Immunol.*, 6:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti- angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

[0200] Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

[0201] Techniques for conjugating such therapeutic moiety to antibodies are well known. See, for example, Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev. 62:119-58 (1982).

[0202] Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

[0203] An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

Antibody-albumin fusion

[0204] Antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention include, but are not limited to, antibodies that bind a Therapeutic protein disclosed in the "Therapeutic Protein X" column of Table 1, or a fragment or variant thereof.

[0205] In specific embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VH domain. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, one, two or three VH CDRs. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VH CDR1. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VH CDR2. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VH CDR3.

[0206] In specific embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VL domain. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, one, two or three VL CDRs. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an

albumin fusion protein comprises, or alternatively consists of, the VL CDR1. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VL CDR2. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VL CDR3.

[0207] In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, one, two, three, four, five, or six VH and/or VL CDRs.

[0208] In preferred embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, an scFv comprising the VH domain of the Therapeutic antibody, linked to the VL domain of the therapeutic antibody by a peptide linker such as $(Gly_4Ser)_3$ (SEQ ID NO:4).

Immunophenotyping

[0209] The antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein (or fragment or variant thereof) may be utilized for immunophenotyping of cell lines and biological samples. Therapeutic proteins of the present invention may be useful as cell-specific markers, or more specifically as cellular markers that are differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies (or albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies (or albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al.*, *Cell*, *96*:737-49 (1999)).

[0210] These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

Characterizing Antibodies that bind a Therapeutic Protein and Albumin Fusion Proteins Comprising a Fragment or Variant of an Antibody that binds a Therapeutic Protein

[0211] The antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein (or fragment or variant thereof) may be characterized in a variety of ways. In particular, Albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be assayed for the ability to specifically bind to the same antigens specifically bound by the antibody that binds a Therapeutic protein corresponding to the antibody that binds a Therapeutic protein portion of the albumin fusion protein using techniques described herein or routinely modifying techniques known in the art.

[0212] Assays for the ability of the antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein (or fragment or variant thereof) to (specifically) bind a specific protein or epitope may be performed in solution (e.g., Houghten, Bio/Techniques 13:412-421(1992)), on beads (e.g., Lam, Nature 354:82-84 (1991)), on chips (e.g., Fodor, Nature 364:555-556 (1993)), on bacteria (e.g., U.S. Patent No. 5,223,409), on spores (e.g., Patent Nos. 5,571,698; 5,403,484; and 5,223,409), on plasmids (e.g., Cull et al., Proc. Natl. Acad. Sci. USA 89:1865-1869 (1992)) or on phage (e.g., Scott and Smith, Science 249:386-390 (1990); Devlin, Science 249:404-406 (1990); Cwirla et al., Proc. Natl. Acad. Sci. USA 87:6378-6382 (1990); and Felici, J. Mol. Biol. 222:301-310 (1991)) (each of these references is incorporated herein in its entirety by reference). The antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein (or fragment or variant thereof) may also be assayed for their specificity and affinity for a specific protein or epitope using or routinely modifying techniques described herein or otherwise known in the art.

[0213] The albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be assayed for cross-reactivity with other antigens (e.g., molecules that have sequence/structure conservation with the molecule(s) specifically bound by the antibody that binds a Therapeutic protein (or fragment or variant thereof) corresponding to the Therapeutic protein portion of the albumin fusion protein of the invention) by any method known in the art.

[0214] Immunoassays which can be used to analyze (immunospecific) binding and cross-reactivity include, but are not limited to, competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below

(but are not intended by way of limitation).

[0215] Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding an antibody of the invention or albumin fusion protein of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein (or fragment or variant thereof) to the cell lysate, incubating for a period of time (e.g., 1 to 4 hours) at 40 degrees C, adding protein A and/or protein G sepharose beads (or beads coated with an appropriate anti-idiotypic antibody or anti-albumin antibody in the case when an albumin fusion protein comprising at least a fragment or variant of a Therapeutic antibody) to the cell lysate, incubating for about an hour or more at 40 degrees C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody or albumin fusion protein of the invention to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody or albumin fusion protein to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

[0216] Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), applying the antibody or albumin fusion protein of the invention (diluted in blocking buffer) to the membrane, washing the membrane in washing buffer, applying a secondary antibody (which recognizes the albumin fusion protein, e.g., an anti-human serum albumin antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., ^{32}P or ^{125}I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

[0217] ELISAs comprise preparing antigen, coating the well of a 96-well microtiter plate with the antigen, washing away antigen that did not bind the wells, adding the antibody or albumin fusion protein (comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) of the invention conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the wells and incubating for a period of time, washing away unbound or non-specifically bound albumin fusion proteins, and detecting the presence of the antibody or albumin fusion proteins specifically bound to the antigen coating the well. In ELISAs the antibody or albumin fusion protein does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody or albumin fusion protein, respectively) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, antibody or the albumin fusion protein may be coated to the well. In this case, the detectable molecule could be the antigen conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase). One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

[0218] The binding affinity of an albumin fusion protein to a protein, antigen, or epitope and the off-rate of an antibody- or albumin fusion protein-protein/antigen/epitope interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., ^{3}H or ^{125}I) with the antibody or albumin fusion protein of the invention in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody or albumin fusion protein of the invention for a specific protein, antigen, or epitope and the binding off-rates can be determined from the data by Scatchard plot analysis. Competition with a second protein that binds the same protein, antigen or epitope as the antibody or albumin fusion protein, can also be determined using radioimmunoassays. In this case, the protein, antigen or epitope is incubated with an antibody or albumin fusion protein of the invention conjugated to a labeled compound (e.g., ^{3}H or ^{125}I) in the presence of increasing amounts of an unlabeled second protein that binds the same protein, antigen, or epitope as the albumin fusion protein of the invention.

[0219] In a preferred embodiment, BIAcore kinetic analysis is used to determine the binding on and off rates of antibody or albumin fusion proteins of the invention to a protein, antigen or epitope. BIAcore kinetic analysis comprises analyzing the binding and dissociation of antibodies, albumin fusion proteins, or specific polypeptides, antigens or epitopes from chips with immobilized specific polypeptides, antigens or epitopes, antibodies or albumin fusion proteins, respectively, on their surface.

Therapeutic Uses

[0220] The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds

of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein), nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein), albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, and nucleic acids encoding such albumin fusion proteins. The antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a Therapeutic protein, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a Therapeutic protein includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

[0221] In a specific and preferred embodiment, the present invention is directed to antibody-based therapies which involve administering antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein to an animal, preferably a mammal, and most preferably a human, patient for treating one or more diseases, disorders, or conditions, including but not limited to: neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions., and/or as described elsewhere herein. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (e.g., antibodies directed to the full length protein expressed on the cell surface of a mammalian cell; antibodies directed to an epitope of a Therapeutic protein and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a Therapeutic protein, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a Therapeutic protein includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

[0222] A summary of the ways in which the antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be used therapeutically includes binding Therapeutic proteins locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein for diagnostic, monitoring or therapeutic purposes without undue experimentation.

[0223] The antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

[0224] The antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

[0225] It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against Therapeutic proteins, fragments or regions thereof, (or the albumin fusion protein correlate of such an antibody) for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include dissociation constants or Kd's less than 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M. More preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M or 10^{-8} M. Even more preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

Gene Therapy

[0226] In a specific embodiment, nucleic acids comprising sequences encoding antibodies that bind therapeutic proteins or albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a Therapeutic protein, by way of gene therapy. Gene therapy refers to

therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

[0227] Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described in more detail elsewhere in this application.

Demonstration of Therapeutic or Prophylactic Activity

[0228] The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then *in vivo* for the desired therapeutic or prophylactic activity, prior to use in humans. For example, *in vitro* assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

Therapeutic/Prophylactic Administration and Composition

[0229] The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention. In a preferred embodiment, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

[0230] Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

[0231] Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0232] In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

[0233] In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., In Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid.)

[0234] In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J.Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, e.g., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

[0235] Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

[0236] In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g., Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)),

etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

[0237] The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

[0238] In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0239] The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0240] The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a Therapeutic protein can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[0241] For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

Diagnosis and Imaging

[0242] Labeled antibodies and derivatives and analogs thereof that bind a Therapeutic protein (or fragment or variant thereof) (including albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein), can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of Therapeutic protein. The invention provides for the detection of aberrant expression of a Therapeutic protein, comprising (a) assaying the expression of the Therapeutic protein in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed Therapeutic protein expression level compared to the standard expression level is indicative of aberrant expression.

[0243] The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the Therapeutic protein in cells or body fluid of an individual using one or more antibodies specific to the Therapeutic protein or albumin fusion proteins comprising at least a fragment or variant of an antibody specific to a Therapeutic protein, and (b) comparing the level of gene expression with a standard gene expression

level, whereby an increase or decrease in the assayed Therapeutic protein gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

[0244] Antibodies of the invention or albumin fusion proteins comprising at least a fragment of variant of an antibody specific to a Therapeutic protein can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen et al., J. Cell . Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0245] One facet of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a Therapeutic protein in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the Therapeutic protein is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the therapeutic protein. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

[0246] It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of 99mTc. The labeled antibody, antibody fragment, or albumin fusion protein comprising at least a fragment or variant of an antibody that binds a Therapeutic protein will then preferentially accumulate at the location of cells which contain the specific Therapeutic protein. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

[0247] Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

[0248] In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

[0249] Presence of the labeled molecule can be detected in the patient using methods known in the art for *in vivo* scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

[0250] In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patent using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI). Antibodies that specifically detect the albumin fusion protein but not albumin or the therapeutic protein alone are a preferred embodiment. These can be used to detect the albumin fusion protein as described throughout the specification.

Kits

[0251] The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate

such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

[0252] In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

[0253] In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

[0254] In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

[0255] In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

[0256] The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

[0257] Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

Albumin Fusion Proteins

[0258] The present invention relates generally to albumin fusion proteins and methods of treating, preventing, or ameliorating diseases or disorders. As used herein, "albumin fusion protein" refers to a protein formed by the fusion of at least one molecule of albumin (or a fragment or variant thereof) to at least one molecule of a Therapeutic protein (or fragment or variant thereof). An albumin fusion protein of the invention comprises at least a fragment or variant of a Therapeutic protein and at least a fragment or variant of human serum albumin, which are associated with one another, preferably by genetic fusion (i.e., the albumin fusion protein is generated by translation of a nucleic acid in which a polynucleotide encoding all or a portion of a Therapeutic protein is joined in-frame with a polynucleotide encoding all or a portion of albumin) or to one another. The Therapeutic protein and albumin protein, once part of the albumin fusion protein, may each be referred to as a "portion", "region" or "moiety" of the albumin fusion protein.

[0259] In a preferred embodiment, the invention provides an albumin fusion protein encoded by a polynucleotide or albumin fusion construct described in Table 1 or Table 2. Polynucleotides encoding these albumin fusion proteins are also encompassed by the invention.

[0260] Preferred albumin fusion proteins of the invention, include, but are not limited to, albumin fusion proteins encoded by a nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide encoding at least one molecule of albumin (or a fragment or variant thereof) joined in frame to at least one polynucleotide encoding at least one molecule of a Therapeutic protein (or fragment or variant thereof); a nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide encoding at least one molecule of albumin (or a fragment or variant thereof) joined in frame to at least one polynucleotide encoding at least one molecule of a Therapeutic protein (or fragment or variant thereof) generated as described in Table 1, Table 2 or in the Examples; or a nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide encoding at least one molecule of albumin (or a fragment or variant thereof) joined in frame to at least one polynucleotide encoding at least one molecule of a Therapeutic protein (or fragment or variant thereof), further comprising, for example, one or more of the following elements: (1) a functional self-replicating vector (including but not limited to, a shuttle vector, an expression vector, an integration vector, and/or a replication system), (2) a region for initiation of transcription (e.g., a promoter region, such as for example, a regulatable or inducible promoter, a constitutive promoter), (3) a region

for termination of transcription, (4) a leader sequence, and (5) a selectable marker.

[0261] In one embodiment, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a Therapeutic protein (e.g., as described in Table 1) and a serum albumin protein. In other embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a biologically active and/or therapeutically active fragment of a Therapeutic protein and a serum albumin protein. In other embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a biologically active and/or therapeutically active variant of a Therapeutic protein and a serum albumin protein. In preferred embodiments, the serum albumin protein component of the albumin fusion protein is the mature portion of serum albumin.

[0262] In further embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a Therapeutic protein, and a biologically active and/or therapeutically active fragment of serum albumin. In further embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a Therapeutic protein and a biologically active and/or therapeutically active variant of serum albumin. In preferred embodiments, the Therapeutic protein portion of the albumin fusion protein is the mature portion of the Therapeutic protein.

[0263] In further embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a biologically active and/or therapeutically active fragment or variant of a Therapeutic protein and a biologically active and/or therapeutically active fragment or variant of serum albumin. In preferred embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, the mature portion of a Therapeutic protein and the mature portion of serum albumin.

[0264] Preferably, the albumin fusion protein comprises HA as the N-terminal portion, and a Therapeutic protein as the C-terminal portion. Alternatively, an albumin fusion protein comprising HA as the C-terminal portion, and a Therapeutic protein as the N-terminal portion may also be used.

[0265] In other embodiments, the albumin fusion protein has a Therapeutic protein fused to both the N-terminus and the C-terminus of albumin. In a preferred embodiment, the Therapeutic proteins fused at the N- and C- terminal are the same Therapeutic proteins. In an alternative preferred embodiment, the Therapeutic proteins fused at the N- and C- termini are different Therapeutic proteins. In another preferred embodiment, the Therapeutic proteins fused at the N- and C- termini are different Therapeutic proteins which may be used to treat or prevent the same or a related disease, disorder, or condition (e.g. as listed in the "Preferred Indication Y" column of Table 1). In another preferred embodiment, the Therapeutic proteins fused at the N- and C- termini are different Therapeutic proteins which may be used to treat, ameliorate, or prevent diseases or disorders (e.g. as listed in the "Preferred Indication Y" column of Table 1) which are known in the art to commonly occur in patients simultaneously, concurrently, or consecutively, or which commonly occur in patients in association with one another.

[0266] Albumin fusion proteins of the invention encompass proteins containing one, two, three, four, or more molecules of a given Therapeutic protein X or variant thereof fused to the N- or C- terminus of an albumin fusion protein of the invention, and/or to the N- and/or C- terminus of albumin or variant thereof. Molecules of a given Therapeutic protein X or variants thereof may be in any number of orientations, including, but not limited to, a 'head to head' orientation (e.g., wherein the N-terminus of one molecule of a Therapeutic protein X is fused to the N-terminus of another molecule of the Therapeutic protein X), or a 'head to tail' orientation (e.g., wherein the C-terminus of one molecule of a Therapeutic protein X is fused to the N-terminus of another molecule of Therapeutic protein X).

[0267] In one embodiment, one, two, three, or more tandemly oriented Therapeutic protein X polypeptides (or fragments or variants thereof) are fused to the N- or C- terminus of an albumin fusion protein of the invention, and/or to the N- and/or C- terminus of albumin or variant thereof.

[0268] Albumin fusion proteins of the invention further encompass proteins containing one, two, three, four, or more molecules of a given Therapeutic protein X or variant thereof fused to the N- or C- terminus of an albumin fusion protein of the invention, and/or to the N- and/or C- terminus of albumin or variant thereof, wherein the molecules are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Albumin fusion proteins comprising multiple Therapeutic protein X polypeptides separated by peptide linkers may be produced using conventional recombinant DNA technology. Linkers are particularly important when fusing a small peptide to the large HSA molecule. The peptide itself can be a linker by fusing tandem copies of the peptide or other known linkers can be used. Constructs that incorporate linkers are described in Table 2 or are apparent when examining SEQ ID NO:Y.

[0269] Further, albumin fusion proteins of the invention may also be produced by fusing a Therapeutic protein X or variants thereof to the N-terminal and/or C-terminal of albumin or variants thereof in such a way as to allow the formation of intramolecular and/or intermolecular multimeric forms. In one embodiment of the invention, albumin fusion proteins may be in monomeric or multimeric forms (i.e., dimers, trimers, tetramers and higher multimers). In a further embodiment of the invention, the Therapeutic protein portion of an albumin fusion protein may be in monomeric form or multimeric form (i.e., dimers, trimers, tetramers and higher multimers). In a specific embodiment, the Therapeutic protein portion of an albumin fusion protein is in multimeric form (i.e., dimers, trimers, tetramers and higher multimers), and the albumin protein portion is in monomeric form.

[0270] In addition to albumin fusion protein in which the albumin portion is fused N- terminal and/or C-terminal of the Therapeutic protein portion, albumin fusion proteins of the invention may also be produced by inserting the Therapeutic protein or peptide of interest (e.g., a Therapeutic

protein X as disclosed in Table 1, or an antibody that binds a Therapeutic protein or a fragment or variant thereof) into an internal region of HA. For instance, within the protein sequence of the HA molecule a number of loops or turns exist between the end and beginning of α-helices, which are stabilized by disulphide bonds. The loops, as determined from the crystal structure of HA (PDB identifiers 1AO6, 1BJ5, 1BKE, 1BM0, 1E7E to 1E7I and 1UOR) for the most part extend away from the body of the molecule. These loops are useful for the insertion, or internal fusion, of therapeutically active peptides, particularly those requiring a secondary structure to be functional, or Therapeutic proteins, to essentially generate an albumin molecule with specific biological activity.

[0271] *Loops in human albumin structure into which peptides or polypeptides may be inserted to generate albumin fusion proteins of the* invention include: Val54-Asn61, Thr76-Asp89, Ala92-Glu100, Gln170-Ala176, His 247 - Glu252, Glu 266 - Glu277, Glu 280-His288, Ala362-Glu368, Lys439-Pro447, Val462-Lys475, Thr478-Pro486, and Lys560-Thr566. In more preferred embodiments, peptides or polypeptides are inserted into the Val54-Asn61, Gln170-Ala176, and/or Lys560-Thr566 loops of mature human albumin (SEQ ID NO:1).

[0272] Peptides to be inserted may be derived from either phage display or synthetic peptide libraries screened for specific biological activity or from the active portions of a molecule with the desired function. Additionally, random peptide libraries may be generated within particular loops or by insertions of randomized peptides into particular loops of the HA molecule and in which all possible combinations of amino acids are represented.

[0273] Such library(s) could be generated on HA or domain fragments of HA by one of the following methods:

randomized mutation of amino acids within one or more peptide loops of HA or HA domain fragments. Either one, more or all the residues within a loop could be mutated in this manner;

replacement of, or insertion into one or more loops of HA or HA domain fragments (*i.e.*, internal fusion) of a randomized peptide(s) of length X_n (where X is an amino acid and n is the number of residues;

N-, C- or N- and C- terminal peptide/protein fusions in addition to (a) and/or (b).

[0274] The HA or HA domain fragment may also be made multifunctional by grafting the peptides derived from different screens of different loops against different targets into the same HA or HA domain fragment.

[0275] In preferred embodiments, peptides inserted into a loop of human serum albumin are peptide fragments or peptide variants of the Therapeutic proteins disclosed in Table 1. More particularly, the invention encompasses albumin fusion proteins which comprise peptide fragments or peptide variants at least 7 at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 35, or at least 40 amino acids in length inserted into a loop of human serum albumin. The invention also encompasses albumin fusion proteins which comprise peptide fragments or peptide variants at least 7 at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 35, or at least 40 amino acids fused to the N-terminus of human serum albumin. The invention also encompasses albumin fusion proteins which comprise peptide fragments or peptide variants at least 7 at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 35, or at least 40 amino acids fused to the C-terminus of human serum albumin. For example, short peptides described in Table 1 and 2 (e.g., Therapeutic Y) can be inserted into the albumin loops.

[0276] Generally, the albumin fusion proteins of the invention may have one HA-derived region and one Therapeutic protein-derived region. Multiple regions of each protein, however, may be used to make an albumin fusion protein of the invention. Similarly, more than one Therapeutic protein may be used to make an albumin fusion protein of the invention. For instance, a Therapeutic protein may be fused to both the N- and C-terminal ends of the HA. In such a configuration, the Therapeutic protein portions may be the same or different Therapeutic protein molecules. The structure of bifunctional albumin fusion proteins may be represented as: X-HA-Y or Y-HA-X.

[0277] For example, an anti-BLyS™ scFv-HA-IFNα-2b fusion may be prepared to modulate the immune response to IFNα-2b by anti-BLyS™ scFv. An alternative is making a bi (or even multi) functional dose of HA-fusions *e.g.* HA-IFNα-2b fusion mixed with HA-anti-BLyS™ scFv fusion or other HA-fusions in various ratio's depending on function, half-life etc.

[0278] Bi- or multi-functional albumin fusion proteins may also be prepared to target the Therapeutic protein portion of a fusion to a target organ or cell type via protein or peptide at the opposite terminus of HA.

[0279] As an alternative to the fusion of known therapeutic molecules, the peptides could be obtained by screening libraries constructed as fusions to the N-, C- or N- and C- termini of HA, or domain fragment of HA, of typically 6, 8, 12, 20 or 25 or X_n (where X is an amino acid (aa) and n equals the number of residues) randomised amino acids, and in which all possible combinations of amino acids were represented. A particular advantage of this approach is that the peptides may be selected *in situ* on the HA molecule and the properties of the peptide would therefore be as selected for rather than, potentially, modified as might be the case for a peptide derived by any other method then being attached to HA.

[0280] Additionally, the albumin fusion proteins of the invention may include a linker peptide between the fused portions to provide greater physical separation between the moieties and thus maximize the accessibility of the Therapeutic protein portion, for instance, for binding to its cognate receptor. The linker peptide may consist of amino acids such that it is flexible or more rigid.

[0281] The linker sequence may be cleavable by a protease or chemically to yield the growth hormone related moiety. Preferably, the protease is one which is produced naturally by the host, for example the *S. cerevisiae* protease *kex2* or equivalent proteases.

[0282] Therefore, as described above, the albumin fusion proteins of the invention may have the following formula R1-L-R2; R2-L-R1; or R1-L-R2-L-R1, wherein R1 is at least one Therapeutic protein, peptide or polypeptide sequence, and not necessarily the same Therapeutic protein, L is a linker and R2 is a serum albumin sequence.

[0283] In preferred embodiments, albumin fusion proteins of the invention comprising a Therapeutic protein have a higher plasma stability compared to the plasma stability of the same Therapeutic protein when not fused to albumin. Plasma stability typically refers to the time period between when the Therapeutic protein is administered in vivo and carried into the bloodstream and when the therapeutic protein is degraded and cleared from the bloodstream, into an organ, such as the kidney or liver, that ultimately clears the Therapeutic protein from the body. Plasma stability is calculated in terms of the half-life of the Therapeutic protein in the bloodstream. The half-life of the Therapeutic protein in the bloodstream can be readily determined by common assays known in the art.

[0284] In preferred embodiments, Albumin fusion proteins of the invention comprising a Therapeutic protein have extended shelf life compared to the shelf life the same Therapeutic protein when not fused to albumin. Shelf-life typically refers to the time period over which the therapeutic activity of a Therapeutic protein in solution or in some other storage formulation, is stable without undue loss of therapeutic activity. Many of the Therapeutic proteins are highly labile in their unfused state. As described below, the typical shelf-life of these Therapeutic proteins is markedly prolonged upon incorporation into the albumin fusion protein of the invention.

[0285] Albumin fusion proteins of the invention with "prolonged" or "extended" shelf-life exhibit greater therapeutic activity relative to a standard that has been subjected to the same storage and handling conditions. The standard may be the unfused full-length Therapeutic protein. When the Therapeutic protein portion of the albumin fusion protein is an analog, a variant, or is otherwise altered or does not include the complete sequence for that protein, the prolongation of therapeutic activity may alternatively be compared to the unfused equivalent of that analog, variant, altered peptide or incomplete sequence. As an example, an albumin fusion protein of the invention may retain greater than about 100% of the therapeutic activity, or greater than about 105%, 110%, 120%, 130%, 150% or 200% of the therapeutic activity of a standard when subjected to the same storage and handling conditions as the standard when compared at a given time point.

[0286] Shelf-life may also be assessed in terms of therapeutic activity remaining after storage, normalized to therapeutic activity when storage began. Albumin fusion proteins of the invention with prolonged or extended shelf-life as exhibited by prolonged or extended therapeutic activity may retain greater than about 50% of the therapeutic activity, about 60%, 70%, 80%, or 90% or more of the therapeutic activity of the equivalent unfused Therapeutic protein when subjected to the same conditions.

Expression of Fusion Proteins

[0287] The albumin fusion proteins of the invention may be produced as recombinant molecules by secretion from yeast, a microorganism such as a bacterium, or a human or animal cell line. Preferably, the polypeptide is secreted from the host cells.

[0288] A particular embodiment of the invention comprises a DNA construct encoding a signal sequence effective for directing secretion in yeast, particularly a yeast-derived signal sequence (especially one which is homologous to the yeast host), and the fused molecule of the first aspect of the invention, there being no yeast-derived pro sequence between the signal and the mature polypeptide.

[0289] The *Saccharomyces cerevisiae* invertase signal is a preferred example of a yeast-derived signal sequence.

[0290] Conjugates of the kind prepared by Poznansky *et al.*, (FEBS Lett. 239:18 (1988)), in which separately-prepared polypeptides are joined by chemical cross-linking, are not contemplated.

[0291] The present invention also includes a cell, preferably a yeast cell transformed to express an albumin fusion protein of the invention. In addition to the transformed host cells themselves, the present invention also contemplates a culture of those cells, preferably a monoclonal (clonally homogeneous) culture, or a culture derived from a monoclonal culture, in a nutrient medium. If the polypeptide is secreted, the medium will contain the polypeptide, with the cells, or without the cells if they have been filtered or centrifuged away. Many expression systems are known and may be used, including bacteria (for example *E. coli* and *Bacillus subtilis*), yeasts (for example *Saccharomyces cerevisiae*, *Kluyveromyces lactis* and *Pichia pastoris*, filamentous fungi (for example *Aspergillus*), plant cells, animal cells and insect cells.

[0292] Preferred yeast strains to be used in the production of albumin fusion proteins are D88, DXY1 and BXP10. D88 [*leu2-3, leu2-122, can1, pra1, ubc4*] is a derivative of parent strain AH22*his*$^+$ (also known as DB1; see, e.g., Sleep *et al.* Biotechnology 8:42-46 (1990)). The strain contains a *leu2* mutation which allows for auxotropic selection of 2 micron-based plasmids that contain the LEU2 gene. D88 also exhibits a derepression of PRB1 in glucose excess. The PRB1 promoter is normally controlled by two checkpoints that monitor glucose levels and growth stage. The promoter is activated in wild type yeast upon glucose depletion and entry into stationary phase. Strain D88 exhibits the repression by glucose but maintains the induction upon entry into stationary phase. The PRA1 gene encodes a yeast vacuolar protease, YscA endoprotease A, that is localized in the ER. The UBC4 gene is in the ubiquitination pathway and is involved in targeting short lived and abnormal proteins for ubiquitin dependent degradation. Isolation of this ubc4 mutation was found to increase the copy number of an expression plasmid in the cell and cause an increased level of expression of a desired protein expressed from the plasmid (see, e.g., International Publication No. WO99/00504, hereby incorporated in its

entirety by reference herein).

[0293] DXY1, a derivative of D88, has the following genotype: [*leu2-3, leu2-122, can1, pra1, ubc4, ura3::yap3*]. In addition to the mutations isolated in D88, this strain also has a knockout of the YAP3 protease. This protease causes cleavage of mostly di-basic residues (RR, RK, KR, KK) but can also promote cleavage at single basic residues in proteins. Isolation of this yap3 mutation resulted in higher levels of full length HSA production (see, e.g., U.S. Patent No. 5,965,386 and Kerry-Williams et al., Yeast 14:161-169 (1998), hereby incorporated in their entireties by reference herein).

[0294] BXP10 has the following genotype: *leu2-3, leu2-122, can1, pra1, ubc4, ura3, yap3::URA3, lys2, hsp150::LYS2, pmt1::URA3*. In addition to the mutations isolated in DXY1, this strain also has a knockout of the PMT1 gene and the HSP150 gene. The PMT1 gene is a member of the evolutionarily conserved family of dolichyl-phosphate-D-mannose protein O-mannosyltransferases (Pmts). The transmembrane topology of Pmt1p suggests that it is an integral membrane protein of the endoplasmic reticulum with a role in O-linked glycosylation. This mutation serves to reduce/eliminate O-linked glycosylation of HSA fusions (see, e.g., International Publication No. WO00/44772, hereby incorporated in its entirety by reference herein). Studies revealed that the Hsp150 protein is inefficiently separated from rHA by ion exchange chromatography. The mutation in the HSP150 gene removes a potential contaminant that has proven difficult to remove by standard purification techniques. See, e.g., U.S. Patent No. 5,783,423, hereby incorporated in its entirety by reference herein.

[0295] The desired protein is produced in conventional ways, for example from a coding sequence inserted in the host chromosome or on a free plasmid. The yeasts are transformed with a coding sequence for the desired protein in any of the usual ways, for example electroporation. Methods for transformation of yeast by electroporation are disclosed in Becker & Guarente (1990) *Methods Enzymol.* 194, 182.

[0296] Successfully transformed cells, *i.e.*, cells that contain a DNA construct of the present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an expression construct can be grown to produce the desired polypeptide. Cells can be harvested and lysed and their DNA content examined for the presence of the DNA using a method such as that described by Southern (1975) *J. Mol. Biol.* 98, 503 or Berent *et al.* (1985) *Biotech.* 3, 208. Alternatively, the presence of the protein in the supernatant can be detected using antibodies.

[0297] Useful yeast plasmid vectors include pRS403-406 and pRS413-416 and are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast Integrating plasmids (YIps) and incorporate the yeast selectable markers HIS3, TRP1, LEU2 and URA3. Plasmids pRS413-416 are Yeast Centromere plasmids (Ycps).

[0298] Preferred vectors for making albumin fusion proteins for expression in yeast include pPPC0005, pScCHSA, pScNHSA, and pC4:HSA which are described in detail in Example 1. Figure 2 shows a map of the pPPC0005 plasmid that can be used as the base vector into which polynucleotides encoding Therapeutic proteins may be cloned to form HA-fusions. It contains a *PRB1 S. cerevisiae* promoter (PRB1p), a Fusion leader sequence (FL), DNA encoding HA (rHA) and an *ADH1 S. cerevisiae* terminator sequence. The sequence of the fusion leader sequence *consists of the first 19 amino acids of the signal peptide of human serum albumin (SEQ ID NO:3) and the last five amino acids of the mating factor* alpha 1 *promoter* (SLDKR, see EP-A-387 319 which is hereby incorporated by reference in its entirety).

[0299] The plasmids, pPPC0005, pScCHSA, pScNHSA, and pC4:HSA were deposited on April 11, 2001 at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209 and given accession numbers ATCC PTA-3278, PTA-3276, PTA-3279, and PTA-3277, respectively. Another vector useful for expressing an albumin fusion protein in yeast the pSAC35 vector which is described in Sleep *et al.*, BioTechnology 8:42 (1990) which is hereby incorporated by reference in its entirety.

[0300] A yeast promoter that can be used to express the albumin fusion protein is the MET25 promoter. See, for example, Dominik Mumberg, Rolf Muller and Martin Funk. Nucleic Acids Research, 1994, Vol. 22, No. 25, pp. 5767-5768. The Met25 promoter is 383 bases long (bases –382 to –1) and the genes expressed by this promoter are also known as Met15, Met17, and YLR303W. A preferred embodiment uses the sequence below, where, at the 5' end of the sequence below, the Not I site used in the cloning is underlined and at the 3' end, the ATG start codon is underlined:

GCGGCCGCCGGATGCAAGGGTTCGAATCCCTTAGCTCTCATTATTTTTTGCTTTTTCTCTTGAGGTCACATGATCGCAAAATGGCAAA
TGGCACGTGAAGCTGTCGATATTGGGGAACTGTGGTGGTTGGCAAATGACTAATTAAGTTAGTCAAGGCGCCATCCTCATGAAAACT
GTGTAACATAATAACCGAAGTGTCGAAAAGGTGGCACCTTGTCCAATTGAACACGCTCGATGAAAAAAATAAGATATATATAAGGTT
AAGTAAAGCGTCTGTTAGAAAGGAAGTTTTTCCTTTTTCTTGCTCTCTTGTCTTTTCATCTACTATTTCCTTCGTGTAATACAGGGTCGT
CAGATACATAGATACAATTCTATTACCCCCATCCATACAATG (SEQ ID NO:5)

[0301] Additional promoters that can be used to express the albumin fusion protein in yeast include the following:

a) the cbh1 promoter:

TCTAGAGTTGTGAAGTCGGTAATCCCGCTGTATAGTAATACGAGTGGCATCTAAATACTCCGAAGCTGCTGCGAACCCGGA
GAATCGAGATGTGCTGGAAAGCTTCTAGCGAGCGGCTAAATTAGCATGAAAGGCTATGAGAAATTCTGGAGACGGCTTGT
TGAATCATGGCGTTCCATTCTTCGACAAGCAAAGCGTTCCGTCGCAGTAGCAGGCACTCATTCCCGAAAAAACTCGGAGA
TTCCTAAGTAGCGATGGAACCGGAATAATATAATAGGCAATACATTGAGTTGCCTCGACGGTTGCAATGCAGGGGTACTG

AGCTTGGACATAACTGTTCCGTACCCCACCTCTTCTCAACCTTTGGCGTTTCCCTGATTCAGCGTACCCGTACAAGTCGTAA
TCACTATTAACCCAGACTGACCGGACGTGTTTTGCCCTTCATTTGGAGAAATAATGTCATTGCGATGTGTAATTTGCCTGCT
TGACCGACTGGGGCTGTTCGAAGCCCGAATGTAGGATTGTTATCCGAACTCTGCTCGTAGAGGCATGTTGTGAATCTGTGT
CGGGCAGGACACGCCTCGAAGGTTCACGGCAAGGGAAACCACCGATAGCAGTGTCTAGTAGCAACCTGTAAAGCCGCAA
TGCAGCATCACTGGAAAATACAAACCAATGGCTAAAAGTACATAAGTTAATGCCTAAAGAAGTCATATACCAGCGGCTAA
TAATTGTACAATCAAGTGGCTAAACGTACCGTAATTTGCCAACGGCTTGTGGGGTTCAGAAGCAACGGCAAAGCCCCAC
TTCCCCACGTTTGTTTCTTCACTCAGTCCAATCTCAGCTGGTGATCCCCCAATTGGGTCGCTTGTTTGTTCCGGTGAAGTGA
AAGAAGACAGAGGTAAGAATGTCTGACTCGGAGCGTTTTGCATACAACCAAGGGCAGTGATGGAAGACAGTGAAATGTT
GACATTCAAGGAGTATTTAGCCAGGGATGCTTGAGTGTATCGTGTAAGGAGGTTTGTCTGCCGATACGACGAATACTGTAT
AGTCACTTCTGGTGAAGTGGTCCATATTGAAATGTAAGTCGGCACTGAACAGGCAAAAGATTGAGTTGAAACTGCCTAAG
ATCTCGGGCCCTCGGGCCTTCGGCCTTTGGGTGTACATGTTTGTGCTCCGGGCAAATGCAAAGTGTGGTAGGATCGAACAC
ACTGCTGCCTTTACCAAGCAGCTGAGGGTATGTGATAGGCAAATGTTCAGGGGCCACTGCATGGTTTCGAATAGAAAGAG
AAGCTTAGCCAAGAACAATAGCCGATAAAGATAGCCTCATTAAACGGAATGAGCTAGTAGGCAAAGTCAGCGAATGTGT
ATATATAAAGGTTCGAGGTCCGTGCCTCCCTCATGCTCTCCCCATCTACTCATCAACTCAGATCCTCCAGGAGACTTGTAC
ACCATCTTTTGAGGCACAGAAACCCAATAGTCAACCGCGGACTGGCATC (SEQ ID NO:113)

b) the cysD promoter from *Aspergillus nidulans*:

AGATCTGGTTCCTGAGTACATCTACCGATGCGCCTCGATCCCCCTCTTAGCCGCATGAGATTCCTACCATTTATGTCCTATCG
TTCAGGGTCCTATTTGGACCGCTAGAAATAGACTCTGCTCGATTTGTTTCCATTATTCACGCAATTACGATAGTATTTGGCTC
TTTTCGTTTGGCCCAGGTCAATTCGGGTAAGACGCGATCACGCCATTGTGGCCGCCGGCGTTGTGCTGCTGCTATTCCCCGC
ATATAAACAACCCCTCCACCAGTTCGTTGGGCTTTGCGAATGCTGTACTCTATTTCAAGTTGTCAAAAGAGAGGATTCAAAA
AATTATACCCCAGATATCAAAGATATCAAAGCCATC (SEQ ID NO:114)

c) a modified cbh1 promoter having the sequence:

TCTAGAGTTGTGAAGTCGGTAATCCCGCTGTATAGTAATACGAGTCGCATCTAAATACTCCGAAGCTGCTGCGAACCCGGA
GAATCGAGATGTGCTGGAAAGCTTCTAGCGAGCGGCTAAATTAGCATGAAAGGCTATGAGAAATTCTGGAGACGGCTTGT
TGAATCATGGCGTTCCATTCTTCGACAAGCAAAGCGTTCCGTCGCAGTAGCAGGCACTCATTCCCGAAAAAACTCGGAGA
TTCCTAAGTAGCGATGGAACCGGAATAATATAATAGGCAATACATTGAGTTGCCTCGACGGTTGCAATGCAGGGGTACTG
AGCTTGGACATAACTGTTCCGTACCCCACCTCTTCTCAACCTTTGGCGTTTCCCTGATTCAGCGTACCCGTACAAGTCGTAA
TCACTATTAACCCAGACTGACCGGACGTGTTTTGCCCTTCATTTGGAGAAATAATGTCATTGCGATGTGTAATTTGCCTGCT
TGACCGACTGGGGCTGTTCGAAGCCCGAATGTAGGATTGTTATCCGAACTCTGCTCGTAGAGGCATGTTGTGAATCTGTGT
CGGGCAGGACACGCCTCGAAGGTTCACGGCAAGGGAAACCACCGATAGCAGTGTCTAGTAGCAACCTGTAAAGCCGCAA
TGCAGCATCACTGGAAAATACAAACCAATGGCTAAAAGTACATAAGTTAATGCCTAAAGAAGTCATATACCAGCGGCTAA
TAATTGTACAATCAAGTGGCTAAACGTACCGTAATTTGCCAACGGCTTGTGGGGTTCAGAAGCAACGGCAAAGCCCCAC
TTCCCCACGTTTGTTTCTTCACTCAGTCCAATCTCAGCTGGTGATCCCCCAATTGGGTCGCTTGTTTGTTCCGGTGAAGTGA
AAGAAGACAGAGGTAAGAATGTCTGACTCGGAGCGTTTTGCATACAACCAAGGGCAGTGATGGAAGACAGTGAAATGTT
GACATTCAAGGAGTATTTAGCCAGGGATGCTTGAGTGTATCGTGTAAGGAGGTTTGTCTGCCGATACGACGAATACTGTAT
AGTCACTTCTGGTGAAGTGGTCCATATTGAAATGTAAGTCGGCACTGAACAGGCAAAAGATTGAGTTGAAACTGCCTAAG
ATCTCGGGCCCTCGGGCCTTCGGCCTTTGGGTGTACATGTTTGTGCTCCGGGCAAATGCAAAGTGTGGTAGGATCGAACAC
ACTGCTGCCTTTACCAAGCAGCTGAGGGTATGTGATAGGCAAATGTTCAGGGGCCACTGCATGGTTTCGAATAGAAAGAG
AAGCTTAGCCTGCAGCCTCTTATCGAGAAAGAAATTACCGTCGCTCGTGATTTGTTTGCAAAAAGAACAAAACTGAAAAA
ACCCAGACACGCTCGACTTCCTGTCTTCCTATTGATTGCAGCTTCCAATTTCGTCACACAACAAGGTCCTAGCTTAGCCAA
GAACAATAGCCGATAAAGATAGCCTCATTAAACCGAATGAGCTAGTAGGCAAAGTCAGCGAATGTGTATATATAAAGGTT
CGAGGTCCGTGCCTCCCTCATGCTCTCCCCATCTACTCATCAACTCAGATCCTCCAGGAGACTTGTACACCATCTTTTGAGG
CACAGAAACCCAATAGTCAACCGCGGACTGGCATC (SEQ ID NO:115)

d) a cysD promoter from *Aspergillus nidulans* having the sequence:

AGATCTGGTTCCTGAGTACATCTACCGATGCGCCTCGATCCCCCTCTTAGCCGCATGAGATTCCTACCATTTATGTCCTATC
GTTCAGGGTCCTATTTGGACCGCTAGAAATAGACTCTGCTCGATTTGTTTCCATTATTCACGCAATTACGATAGTATTTGGC
TCTTTTCGTTTGGCCCAGGTCAATTCGGGTAAGACGCGATCACGCCATTGTGGCCGCCGGCGCTGCAGCCTCTTATCGAGA
AAGAAATTACCGTCGCTCGTGATTTGTTTGCAAAAAGAACAAAACTGAAAAAACCCAGACACGCTCGACTTCCTGTCTTCC
TATTGATTGCAGCTTCCAATTTCGTCACACAACAAGGTCCTACGCCGGCGTTGTGCTGCTGCTATTCCCCGCATATAAACA
ACCCCTCCACCAGTTCGTTGGGCTTTGCGAATGCTGTACTCTATTTCAAGTTGTCAAAAGAGAGGATTCAAAAAATTATAC
CCCAGATATCAAAGATATCAAAGCCATC (SEQ ID NO:116)

[0302] A variety of methods have been developed to operably link DNA to vectors via complementary cohesive termini. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

[0303] Synthetic linkers containing one or more restriction sites provide an alternative method of joining the DNA segment to vectors. The DNA segment, generated by endonuclease restriction digestion, is treated with bacteriophage T4 DNA polymerase or E. coli DNA polymerase I, enzymes that remove protruding, gamma-single-stranded termini with their 3' 5'-exonucleolytic activities, and fill in recessed 3'-ends with their polymerizing activities.

[0304] The combination of these activities therefore generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a large molar excess of linker molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-ended DNA molecules, such as bacteriophage T4 DNA ligase. Thus, the products of the reaction are DNA segments carrying polymeric linker sequences at their ends. These DNA segments are then cleaved with the appropriate restriction enzyme and ligated to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

[0305] Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies Inc, New Haven, CT, USA.

[0306] A desirable way to modify the DNA in accordance with the invention, if, for example, HA variants are to be prepared, is to use the polymerase chain reaction as disclosed by Saiki *et al.* (1988) *Science* 239, 487-491. In this method the DNA to be enzymatically amplified is flanked by two specific oligonucleotide primers which themselves become incorporated into the amplified DNA. The specific primers may contain restriction endonuclease recognition sites which can be used for cloning into expression vectors using methods known in the art.

[0307] Exemplary genera of yeast contemplated to be useful in the practice of the present invention as hosts for expressing the albumin fusion proteins are *Pichia* (Hansenula), *Saccharomyces*, *Kluyveromyces*, *Candida*, *Torulopsis*, *Torulaspora*, *Schizosaccharomyces*, *Citeromyces*, *Pachysolen*, *Debaromyces*, *Metschunikowia*, *Rhodosporidium*, *Leucosporidium*, *Botryoascus*, *Sporidiobolus*, *Endomycopsis*, and the like. Preferred genera are those selected from the group consisting of *Saccharomyces*, *Schizosaccharomyces*, *Kluyveromyces*, *Pichia* and *Torulaspora*. Examples of *Saccharomyces* spp. are *S. cerevisiae*, *S. italicus and S. rouxii.*

[0308] Examples of Kluyveromyces spp. are K. fragilis, K. lactis and K. marxianus. A suitable Torulaspora species is T. delbrueckii. Examples of Pichia (Hansenula) spp. are P. angusta (formerly H. polymorpha), P. anomala (formerly H. anomala) and P. pastoris. Methods for the transformation of S. cerevisiae are taught generally in EP 251 744, EP 258 067 and WO 90/01063, all of which are incorporated herein by reference.

[0309] Preferred exemplary species of Saccharomyces include S. cerevisiae, S. italicus, S. diastaticus, and Zygosaccharomyces rouxii. Preferred exemplary species of Kluyveromyces include K. fragilis and K. lactis. Preferred exemplary species of Hansenula include H. polymorpha (now Pichia angusta), H. anomala (now Pichia anomala), and Pichia capsulata. Additional preferred exemplary species of Pichia include P. pastoris. Preferred exemplary species of Aspergillus include A. *niger* and A. *nidulans*. Preferred exemplary species of Yarrowia include Y. *lipolytica*. Many preferred yeast species are available from the ATCC. For example, the following preferred yeast species are available from the ATCC and are useful in the expression of albumin fusion proteins: Saccharomyces cerevisiae Hansen, teleomorph strain BY4743 yap3 mutant (ATCC Accession No. 4022731); Saccharomyces cerevisiae Hansen, teleomorph strain BY4743 hsp150 mutant (ATCC Accession No. 4021266); Saccharomyces cerevisiae Hansen, teleomorph strain BY4743 pmt1 mutant (ATCC Accession No. 4023792); Saccharomyces cerevisiae Hansen, teleomorph (ATCC Accession Nos. 20626; 44773; 44774; and 62995); Saccharomyces diastaticus Andrews et Gilliland ex van der Walt, teleomorph (ATCC Accession No. 62987); Kluyveromyces lactis (Dombrowski) van der Walt, teleomorph (ATCC Accession No. 76492); Pichia angusta (Teunisson et al.) Kurtzman, teleomorph deposited as Hansenula polymorpha de Morais et Maia, teleomorph (ATCC Accession No. 26012); Aspergillus niger van Tieghem, anamorph (ATCC Accession No. 9029); Aspergillus niger van Tieghem, anamorph (ATCC Accession No. 16404); Aspergillus nidulans (Eidam) Winter, anamorph (ATCC Accession No. 48756); and Yarrowia lipolytica (Wickerham et al.) van der Walt et von Arx, teleomorph (ATCC Accession No. 201847).

[0310] Suitable promoters for *S. cerevisiae* include those associated with the PGK1 gene, GAL1 or GAL10 genes, CYC1, PHO5, TRP1, ADH1, ADH2, the genes for glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, triose phosphate isomerase, phosphoglucose isomerase, glucokinase, alpha-mating factor pheromone, [a mating factor pheromone], the PRB1 promoter, the GUT2 promoter, the GPD1 promoter, and hybrid promoters involving hybrids of parts of 5' regulatory regions with parts of 5' regulatory regions of other promoters or with upstream activation sites (e.g. the promoter of EP-A-258 067).

[0311] Convenient regulatable promoters for use in *Schizosaccharomyces pombe* are the thiamine-repressible promoter from the nmt gene as described by Maundrell (1990) *J. Biol. Chem.* 265, 10857-10864 and the glucose repressible jbp1 gene promoter as described by Hoffman & Winston (1990) *Genetics* 124, 807-816.

[0312] Methods of transforming *Pichia* for expression of foreign genes are taught in, for example, Cregg *et al.* (1993), and various Phillips patents (e.g. US 4 857 467, incorporated herein by reference), and *Pichia* expression kits are commercially available from Invitrogen BV, Leek, Netherlands, and Invitrogen Corp., San Diego, California. Suitable promoters include AOX1 and AOX2. Gleeson *et al.* (1986) J. Gen. Microbiol.

132, 3459-3465 include information on *Hansenula* vectors and transformation, suitable promoters being MOX1 and FMD1; whilst EP 361 991, Fleer *et al.* (1991) and other publications from Rhone-Poulenc Rorer teach how to express foreign proteins in *Kluyveromyces* spp., a suitable promoter being PGK1.

[0313] The transcription termination signal is preferably the 3' flanking sequence of a eukaryotic gene which contains proper signals for transcription termination and polyadenylation. Suitable 3' flanking sequences may, for example, be those of the gene naturally linked to the expression control sequence used, *i.e.* may correspond to the promoter. Alternatively, they may be different in which case the termination signal of the *S. cerevisiae* ADH1 gene is preferred.

[0314] The desired albumin fusion protein may be initially expressed with a secretion leader sequence, which may be any leader effective in the yeast chosen. Leaders useful in yeast include any of the following:

a) the MPIF-1 signal sequence (e.g., amino acids 1-21 of GenBank Accession number AAB51134) MKVSVAALSCLMLVTALGSQA (SEQ ID NO:6)

b) the stanniocalcin signal sequence (MLQNSAVLLLLVISASA, SEQ ID NO:7)

c) the pre-pro region of the HSA signal sequence (e.g., MKWVTFISLLFLFSSAYSRGVFRR, SEQ ID NO:8)

d) the pre region of the HSA signal sequence (e.g., MKWVTFISLLFLFSSAYS, SEQ ID NO:9) or variants thereof, such as, for example, MKWVSFISLLFLFSSAYS, (SEQ ID NO:10)

e) the invertase signal sequence (e.g., MLLQAFLFLLAGFAAKISA, SEQ ID NO:11)

f) the yeast mating factor alpha signal sequence (e.g., MRFPSIFTAVLAFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPFSNSTNNGLLFINTTIASIAAKEEGVSLEKR, SEQ ID NO:12 or MRFPSIFTAVLAFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPFSNSTNNGLLFINTTIASIAAKEEGVSLDKR, SEQ ID NO:12)

g) *K. lactis* killer toxin leader sequence

h) a hybrid signal sequence (e.g., MKWVSFISLLFLFSSAYSRSLEKR, SEQ ID NO:13)

i) an HSA/MFα-1 hybrid signal sequence (also known as HSA/kex2) (e.g., MKWVSFISLLFLFSSAYSRSLDKR, SEQ ID NO:14)

j) a *K. lactis* killer/ MFα-1 fusion leader sequence (e.g., MNIFYIFLFLLSFVQGSLDKR, SEQ ID NO:15)

k) the Immunoglobulin Ig signal sequence (e.g., MGWSCIILFLVATATGVHS, SEQ ID NO:16)

l) the Fibulin B precursor signal sequence (e.g., MERAAPSRRVPLPLLLLGGLALLAAGVDA, SEQ ID NO:17)

m) the clusterin precursor signal sequence (e.g., MMKTLLLFVGLLLTWESGQVLG, SEQ ID NO:18)

n) the insulin-like growth factor-binding protein 4 signal sequence (e.g., MLPLCLVAALLLAAGPGPSLG, SEQ ID NO:19)

o) variants of the pre-pro-region of the HSA signal sequence such as, for example, MKWVSFISLLFLFSSAYSRGVFRR (SEQ ID NO:20), MKWVTFISLLFLFAGVLG (SEQ ID NO:21), MKWVTFISLLFLFSGVLG (SEQ ID NO:22), MKWVTFISLLFLFQGVLG (SEQ ID NO:23), Modified HSA leader HSA #64 - MKWVTFISLLFLFAGVSG (SEQ ID NO:24); Modified HSA leader HSA #66 - MKWVTFISLLFLFQGVSG (SEQ ID NO:25); Modified HSA (A14) leader – MKWVTFISLLFLFAGVSG (SEQ ID NO:26); Modified HSA (S14) leader (also known as modified HSA #65) – MKWVTFISLLFLFSGVSG (SEQ ID NO:27), Modified HSA (G14) leader – MKWVTFISLLFLFGGVSG (SEQ ID NO:28), or MKWVTFISLLFLFQGVLGDLHKS (SEQ ID NO:29)

p) a consensus signal sequence (MPTWAWWLFLVLLALWAPARG, SEQ ID NO:30)

q) acid phosphatase (PH05) leader (e.g., MFKSVVYSILAASLANA SEQ ID NO:31)

r) the pre-sequence of MFα-1

s) the pre-sequence of 0 glucanase (BGL2)

t) killer toxin leader

u) the presequence of killer toxin

v) k. lactis killer toxin prepro (29 amino acids; 16 amino acids of pre and 13 amino acids of pro) MNIFYIFLFLLSFVQGLEHTHRRGSLDKR (SEQ ID NO:32)

w) *S. diastaticus* glucoamylase II secretion leader sequence

x) *S. carlsbergensis* α-galactosidase (MEL1) secretion leader sequence

y) *Candida glucoamylase* leader sequence

z) The hybrid leaders disclosed in EP-A-387 319 (herein incorporated by reference)

aa) the gp67 signal sequence (in conjunction with baculoviral expression systems) (e.g., amino acids 1-19 of GenBank Accession Number AAA72759) or

bb) the natural leader of the therapeutic protein X;

cc) S. cerevisiae invertase (SUC2) leader, as disclosed in JP 62-096086 (granted as 911036516, herein incorporate by reference); or

dd) Inulinase – MKLAYSLLLPLAGVSASVINYKR (SEQ ID NO:33).

ee) A modified TA57 propeptide leader variant #1 – MKLKTVRSAVLSSLFASQVLGQPIDDTESQTTSVNLMADDTESAFATQTNSGGLDVVGLISMAKR (SEQ ID NO:34)

ff) A modified TA57 propeptide leader variant #2 – MKLKTVRSAVLSSLFASQVLGQPIDDTESQTTSVNLMADDTESAFATQTNSGGLDVVGLISMAEEGEPKR (SEQ ID NO:35)

gg) A consensus signal peptide – MWWRLWWLLLLLLLLWPMVWA (SEQ ID NO:111)

hh) A modified HSA/kex2 signal sequence- MKWVSFISLLFLFSSAYSGSLDKR (SEQ ID NO:112)

ii) A consensus signal peptide #2 – MRPTWAWWLFLVLLLALWAPARG (SEQ ID NO:105)

Additional Methods of Recombinant and Synthetic Production of Albumin Fusion Proteins

[0315] The present invention also relates to vectors containing a polynucleotide encoding an albumin fusion protein of the present invention, host cells, and the production of albumin fusion proteins by synthetic and recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

[0316] The polynucleotides encoding albumin fusion proteins of the invention may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

[0317] The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli lac, trp, phoA* and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

[0318] As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418, glutamine synthase, or neomycin resistance for eukaryotic cell culture, and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, Streptomyces and *Salmonella typhimurium* cells; fungal cells, such as yeast cells (e.g., *Saccharomyces cerevisiae* or *Pichia pastoris* (ATCC Accession No. 201178)); insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, NSO, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

[0319] Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

[0320] In one embodiment, polynucleotides encoding an albumin fusion protein of the invention may be fused to signal sequences which will direct the localization of a protein of the invention to particular compartments of a prokaryotic or eukaryotic cell and/or direct the secretion of a protein of the invention from a prokaryotic or eukaryotic cell. For example, in *E. coli*, one may wish to direct the expression of the protein to the periplasmic space. Examples of signal sequences or proteins (or fragments thereof) to which the albumin fusion proteins of the invention may be fused in order to direct the expression of the polypeptide to the periplasmic space of bacteria include, but are not limited to, the *pelB* signal sequence, the maltose binding protein (MBP) signal sequence, MBP, the *ompA* signal sequence, the signal sequence of the periplasmic *E. coli* heat-labile enterotoxin B-subunit, and the signal sequence of alkaline phosphatase. Several vectors are commercially available for the construction of fusion proteins which will direct the localization of a protein, such as the pMAL series of vectors (particularly the pMAL-p series) available from New England Biolabs. In a specific embodiment, polynucleotides albumin fusion proteins of the invention may be fused to the *pelB* pectate lyase signal sequence to increase the efficiency of expression and purification of such polypeptides in Gram-negative bacteria. *See*, U.S. Patent Nos. 5,576,195 and 5,846,818, the contents of which are herein incorporated by reference in their entireties.

[0321] Examples of signal peptides that may be fused to an albumin fusion protein of the invention in order to direct its secretion in mammalian cells include, but are not limited to:

a) the MFP-1 signal sequence (e.g., amino acids 1-21 of GenBank Accession number AAB51134) MKVSVAALSCLMLVTALGSQA (SEQ ID NO:6)

b) the stanniocalcin signal sequence (MLQNSAVLLLLVISASA, SEQ ID NO:7)

c) the pre-pro region of the HSA signal sequence (e.g., MKWVTFISLLFLFSSAYSRGVFRR, SEQ ID NO:8)

d) the pro region of the HSA signal sequence (e.g., MKWVTFISLLFLFSSAYS, SEQ ID NO:9) or variants thereof, such as, for example, MKWVSFISLLFLFSSAYS, (SEQ ID NO:10)

e) the invertase signal sequence (e.g., MLLQAFLFLLAGFAAKISA, SEQ ID NO:11)

f) the yeast mating factor alpha signal sequence (e.g., MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPFSNSTNNGLLFINTTIASIAAKEEGVSLEKR, SEQ ID NO:12 or MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPFSNSTNNGLLFINTTIASIAAKEEGVSLDKR, SEQ ID NO:12)

g) *K. lactis* killer toxin leader sequence

h) a hybrid signal sequence (e.g., MKWVSFISLLFLFSSAYSRSLEKR, SEQ ID NO:13)

i) an HSA/MFα-1 hybrid signal sequence (also known as HSA/hex2) (e.g., MKWVSFISLLFLFSSAYSRSLDKR, SEQ ID NO:14)

j) a *K. lactis* killer/ MFα-1 fusion leader sequence (e.g., MNIFYIFLFLLSFVQGSLDKR, SEQ ID NO:15)

k) the immunoglobulin Ig signal sequence (e.g., MGWSCIILFLVATATGVHS, SEQ ID NO:16)

l) the Fibulin B precursor signal sequence (e.g., MERAAPSRRVPLPLLLLGGLALLAAGVDA, SEQ ID NO:17)

m) the clusterin precursor signal sequence (e.g., MMKTLLLFVGLLLTWESGQVLG, SEQ ID NO:18)

n) the insulin-like growth factor-binding protein 4 signal sequence (e.g., MLPLCLVAALLLAAGPGPSLG, SEQ ID NO:19)

o) variants of the pre-pro-region of the HSA signal sequence such as, for example, MKWVSFISLLFLFSSAYSRGVFRR (SEQ ID NO:20), MKWVTFISLLFLFAGVLG (SEQ ID NO:21), MKWVTFISLLFLFSGVLG (SEQ ID NO:22), MKWVTFISLLFLFGGVLG (SEQ ID NO:23), Modified HSA leader HSA #64 - MKWVTFISLLFLFAGVSG (SEQ ID NO:24); Modified HSA leader HSA #66 - MKWVTFISLLFLFGGVSG (SEQ ID NO:25); Modified HSA (A14) leader – MKWVTFISLLFLFAGVSG (SEQ ID NO:26); Modified HSA (S14) leader (also known as modified HSA #65) – MKWVTFISLLFLFSGVSG (SEQ ID NO:27), Modified HSA (G14) leader – MKWVTFISLLFLFGGVSG (SEQ ID NO:28), or MKWVTFISLLFLFGGVLGDLHKS (SEQ ID NO:29)

p) a consensus signal sequence (MPTWAWWLFLVLLLALWAPARG, SEQ ID NO:30)

q) acid phosphatase (PH05) leader (e.g., MFKSVVYSILAASLANA SEQ ID NO:31)

r) the pre-sequence of MFα-1

s) the pre-sequence of 0 glucanase (BGL2)

t) killer toxin leader

u) the presequence of killer toxin

v) k. lactis killer toxin prepro (29 amino acids; 16 amino acids of pre and 13 amino acids of pro) *MNIFYIFLFLLSFVQGLEHTHRRGSLDKR* (SEQ ID NO:32)

w) *S. diastaticus* glucoamylase II secretion leader sequence

x) *S. carlsbergensis* α-galactosidase (MEL1) secretion leader sequence

y) *Candida glucoamylase* leader sequence

z) The hybrid leaders disclosed in EP-A-387 319 (herin incorporated by reference)

aa) the gp67 signal sequence (in conjunction with baculoviral expression systems) (e.g., amino acids 1-19 of GenBank Accession Number AAA72759) or

bb) the natural leader of the therapeutic protein X;

cc) S. cerevisiae invertase (SUC2) leader, as disclosed in JP 62-096086 (granted as 911036516, herein incorporate by reference); or

dd) Inulinase – MKLAYSLLLPLAGVSASVINYKR (SEQ ID NO:33).

ee) A modified TA57 propeptide leader variant #1 – MKLKTVRSAVLSSLFASQVLGQPIDDTESQTTSVNLMADDTESAFATQTNSGGLDVVGLISMAKR (SEQ ID NO:34)

ff) A modified TA57 propeptide leader variant #2 – MKLKTVRSAVLSSLFASQVLGQPIDDTESQTTSVNLMADDTESAFATQTNSGGLDVVGLISMAEEGEPKR (SEQ ID NO:35)

gg) A consensus signal peptide – MWWRLWWLLLLLLLLWPMVWA (SEQ ID NO:111)

jj) A modified HSA/kex2 signal sequence- MKWVSFISLLFLFSSAYSGSLDKR (SEQ ID NO:112)

kk) A consensus signal peptide #2 – MRPTWAWWLFLVLLLALWAPARG (SEQ ID NO:105)

[0322] In a preferred embodiment, the modified HSA/kex2 signal sequence (SEQ ID NO:112) is fused to the amino terminus of an albumin fusion protein, including fusion proteins comprising albumin and a therapeutic protein as described herein, as well as albumin fusion proteins disclosed in WO93/15199; WO97/24445; WO03/60071; WO03/59934; and PCT/US04/01369, each of which are incorporated herein by reference in their entireties. The modified HSA/kex2 signal sequence is based on the HSA/kex2 signal sequence (SEQ ID NO:14) disclosed, e.g., in Sleep et al., Biotechnology 1990, vol. 8, pp. 42-46; and US Patent 5,302,697, both of which are incorporated herein by reference in their entireties. The modified HSA/kex2 leader sequence disclosed herein contains a non-conservative amino acid substitution (Arg to Gly) at residue 19 of the parent signal peptide. The modified HSA/kex2 signal peptide has been found to produce unexpectedly better expression yield and/or better cleavage efficiency of albumin fusion proteins when expressed in yeast than the unmodified HSA/kex2 signal sequence. Variants of the modified HSA/kex2 signal peptide are also encompassed by the invention. In particular the Gly residue at position 19 of SEQ ID NO:112 may be substituted with a Pro residue. Other conservative substitution variants of the modified HSA/kex2 signal sequence are also contemplated. Nucleic acids encoding the modified HSA/kex2 signal sequence of SEQ ID NO:112, as well as conservative substitution variants thereof, are also encompassed by the invention.

[0323] Vectors which use glutamine synthase (GS) or DHFR as the selectable markers can be amplified in the presence of the drugs methionine sulphoximine or methotrexate, respectively. An advantage of glutamine synthase based vectors are the availability of cell lines (e.g., the murine myeloma cell line, NSO) which are glutamine synthase negative. Glutamine synthase expression systems can also function in glutamine synthase expressing cells (e.g., Chinese Hamster Ovary (CHO) cells) by providing additional inhibitor to prevent the functioning of the endogenous gene. A glutamine synthase expression system and components thereof are detailed in PCT publications: WO87/04462; WO86/05807; WO89/01036; WO89/10404; and WO91/06657, which are hereby incorporated in their entireties by reference herein. Additionally, glutamine synthase expression vectors can be obtained from Lonza Biologics, Inc. (Portsmouth, NH). Expression and production of monoclonal antibodies using a GS expression system in murine myeloma cells is described in Bebbington *et al.*, *Bio/technology* 10:169(1992) and in Biblia and Robinson *Biotechnol. Prog.* 11:1 (1995) which are herein incorporated by reference.

[0324] The present invention also relates to host cells containing the above-described vector constructs described herein, and additionally encompasses host cells containing nucleotide sequences of the invention that are operably associated with one or more heterologous control regions (e.g., promoter and/or enhancer) using techniques known of in the art. The host cell can be a higher eukaryotic cell, such as a mammalian cell (e.g., a human derived cell), or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. A host strain may be chosen which modulates the expression of the inserted gene sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus expression of the genetically engineered polypeptide may be controlled. Furthermore, different host cells have characteristics and specific mechanisms for the translational and post-translational processing and modification (e.g., phosphorylation, cleavage) of proteins. Appropriate cell lines can be chosen to ensure the desired modifications and processing of the foreign protein expressed.

[0325] Introduction of the nucleic acids and nucleic acid constructs of the invention into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods in Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., the coding sequence corresponding to a Therapeutic protein may be replaced with an albumin fusion protein corresponding to the Therapeutic protein), and/or to include genetic material (e.g., heterologous polynucleotide sequences such as for example, an albumin fusion protein of the invention corresponding to the Therapeutic protein may be included). The genetic material operably associated with the endogenous polynucleotide may activate, alter, and/or amplify endogenous polynucleotides.

[0326] In addition, techniques known in the art may be used to operably associate heterologous polynucleotides (e.g., polynucleotides encoding an albumin protein, or a fragment or variant thereof) and/or heterologous control regions (e.g., promoter and/or enhancer) with endogenous polynucleotide sequences encoding a Therapeutic protein via homologous recombination (see, e.g., US Patent Number 5,641,670, issued June 24, 1997; International Publication Number WO 96/29411; International Publication Number WO 94/12650; Koller *et al.*, *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); and Zijlstra *et al.*, *Nature* 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

[0327] Albumin fusion proteins of the invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography,

hydrophobic interaction chromatography, affinity chromatography, hydroxyapatite chromatography, hydrophobic charge interaction chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

[0331] In preferred embodiments the albumin fusion proteins of the invention are purified using Anion Exchange Chromatography including, but not limited to, chromatography on Q-sepharose, DEAE sepharose, poros HQ, poros DEAE, Toyopearl Q, Toyopearl QAE, Toyopearl DEAE, Resource/Source Q and DEAE, Fractogel Q and DEAE columns.

[0332] In specific embodiments the albumin fusion proteins of the invention are purified using Cation Exchange Chromatography including, but not limited to, SP-sepharose, CM sepharose, poros HS, poros CM, Toyopearl SP, Toyopearl CM, Resource/Source S and CM, Fractogel S and CM columns and their equivalents and comparables.

[0333] In specific embodiments the albumin fusion proteins of the invention are purified using Hydrophobic Interaction Chromatography including, but not limited to, Phenyl, Butyl, Methyl, Octyl, Hexyl-sepharose, poros Phenyl, Butyl, Methyl, Octyl, Hexyl, Toyopearl Phenyl, Butyl, Methyl, Octyl, Hexyl Resource/Source Phenyl, Butyl, Methyl, Octyl, Hexyl, Fractogel Phenyl, Butyl, Methyl, Octyl, Hexyl columns and their equivalents and comparables.

[0334] In specific embodiments the albumin fusion proteins of the invention are purified using Size Exclusion Chromatography including, but not limited to, sepharose S100, S200, S300, superdex resin columns and their equivalents and comparables.

[0335] In specific embodiments the albumin fusion proteins of the invention are purified using Affinity Chromatography including, but not limited to, Mimetic Dye affinity, peptide affinity and antibody affinity columns that are selective for either the HSA or the "fusion target" molecules.

[0336] In preferred embodiments albumin fusion proteins of the invention are purified using one or more Chromatography methods listed above. In other preferred embodiments, albumin fusion proteins of the invention are purified using one or more of the following Chromatography columns, Q sepharose FF column, SP Sepharose FF column, Q Sepharose High Performance Column, Blue Sepharose FF column , Blue Column, Phenyl Sepharose FF column, DEAE Sepharose FF, or Methyl Column.

[0337] Additionally, albumin fusion proteins of the invention may be purified using the process described in PCT International Publication WO 00/44772 which is herein incorporated by reference in its entirety. One of skill in the art could easily modify the process described therein for use in the purification of albumin fusion proteins of the invention.

[0338] Albumin fusion proteins of the present invention may be recovered from: products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, albumin fusion proteins of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

[0339] In one embodiment, the yeast *Pichia pastoris* is used to express albumin fusion proteins of the invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolization pathway is the oxidation of methanol to formaldehyde using O_2. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O_2. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. See Ellis, S.B., *et al.*, *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J, *et al.*, *Yeast* 5:167-77 (1989); Tschopp, J.F., *et al.*, *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

[0340] In one example, the plasmid vector pPIC9K is used to express DNA encoding an albumin fusion protein of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a polypeptide of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

[0341] Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

[0342] In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide encoding an

albumin fusion protein of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

[0343] In addition, albumin fusion proteins of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

[0344] The invention encompasses albumin fusion proteins of the present invention which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to, specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

[0345] Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The albumin fusion proteins may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

[0346] Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include iodine (^{121}I, ^{123}I, ^{125}I, ^{131}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{111}In, ^{112}In, ^{113m}In, ^{115m}In), technetium (^{99}Tc, ^{99m}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, and ^{97}Ru.

[0347] In specific embodiments, albumin fusion proteins of the present invention or fragments or variants thereof are attached to macrocyclic chelators that associate with radiometal ions, including but not limited to, ^{177}Lu, ^{90}Y, ^{166}Ho, and ^{153}Sm, to polypeptides. In a preferred embodiment, the radiometal ion associated with the macrocyclic chelators is ^{111}In. In another preferred embodiment, the radiometal ion associated with the macrocyclic chelator is ^{90}Y. In specific embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA). In other specific embodiments, DOTA is attached to an antibody of the invention or fragment thereof via linker molecule. Examples of linker molecules useful for conjugating DOTA to a polypeptide are commonly known in the art - see, for example, DeNardo et al., Clin Cancer Res. 4(10):2483-90 (1998); Peterson et al., Bioconjug. Chem. 10(4):553-7 (1999); and Zimmerman et al, Nucl. Med. Biol. 26(8):943-50 (1999); which are hereby incorporated by reference in their entirety.

[0348] As mentioned, the albumin fusion proteins of the invention may be modified by either natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Polypeptides of the invention may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POST-TRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth. Enzymol. 182:626-646 (1990); Rattan et al., Ann. N.Y. Acad. Sci. 663:48-62 (1992)).

[0349] Albumin fusion proteins of the invention and antibodies that bind a Therapeutic protein or fragments or variants thereof can be fused to

marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

[0350] Further, an albumin fusion protein of the invention may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, 213Bi. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis- dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

[0351] The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, B-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF-alpha, TNF-beta, AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al., Int. Immunol.*, 6:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti- angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors. Techniques for conjugating such therapeutic moiety to proteins (e.g., albumin fusion proteins) are well known in the art.

[0352] Albumin fusion proteins may also be attached to solid supports, which are particularly useful for immunoassays or purification of polypeptides that are bound by, that bind to, or associate with albumin fusion proteins of the invention. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

[0353] Albumin fusion proteins, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

[0354] In embodiments where the albumin fusion protein of the invention comprises only the VH domain of an antibody that binds a Therapeutic protein, it may be necessary and/or desirable to coexpress the fusion protein with the VL domain of the same antibody that binds a Therapeutic protein, such that the VH-albumin fusion protein and VL protein will associate (either covalently or non-covalently) post-translationally.

[0355] In embodiments where the albumin fusion protein of the invention comprises only the VL domain of an antibody that binds a Therapeutic protein, it may be necessary and/or desirable to coexpress the fusion protein with the VH domain of the same antibody that binds a Therapeutic protein, such that the VL-albumin fusion protein and VH protein will associate (either covalently or non-covalently) post-translationally.

[0356] Some Therapeutic antibodies are bispecific antibodies, meaning the antibody that binds a Therapeutic protein is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. In order to create an albumin fusion protein corresponding to that Therapeutic protein, it is possible to create an albumin fusion protein which has an scFv fragment fused to both the N- and C- terminus of the albumin protein moiety. More particularly, the scFv fused to the N-terminus of albumin would correspond to one of the heavy/light (VH/VL) pairs of the original antibody that binds a Therapeutic protein and the scFv fused to the C-terminus of albumin would correspond to the other heavy/light (VH/VL) pair of the original antibody that binds a Therapeutic protein.

[0357] Also provided by the invention are chemically modified derivatives of the albumin fusion proteins of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The albumin fusion proteins may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

[0358] The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the

desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a Therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 45,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

[0359] As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al., Appl. Biochem. Biotechnol. 56*:59-72 (1996); Vorobjev *et al., Nucleosides Nucleotides 18*:2745-2750 (1999); and Caliceti *et al., Bioconjug. Chem. 10*:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

[0360] The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, such as, for example, the method disclosed in EP 0 401 384 (coupling PEG to G-CSF), herein incorporated by reference; see also Malik et al., Exp. Hematol. 20:1028-1035 (1992), reporting pegylation of GM-CSF using tresyl chloride. For example, polyethylene glycol may be covalently bound through amino acid residues via reactive group, such as a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

[0361] As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

[0362] One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

[0363] As indicated above, pegylation of the albumin fusion proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the albumin fusion protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado et al., Crit. Rev. Thera. Drug Carrier Sys. 9:249-304 (1992); Francis et al., Intern. J. of Hematol. 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

[0364] One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ($ClSO_2CH_2CF_3$). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

[0365] Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number of additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in International Publication No. WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

[0366] The number of polyethylene glycol moieties attached to each albumin fusion protein of the invention (i.e., the degree of substitution) may

also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado et al., Crit. Rev. Thera. Drug Carrier Sys. 9:249-304 (1992).

[0367] The polypeptides of the invention can be recovered and purified from chemical synthesis and recombinant cell cultures by standard methods which include, but are not limited to, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and/or purification.

[0368] The presence and quantity of albumin fusion proteins of the invention may be determined using ELISA, a well known immunoassay known in the art. In one ELISA protocol that would be useful for detecting/quantifying albumin fusion proteins of the invention, comprises the steps of coating an ELISA plate with an anti-human serum albumin antibody, blocking the plate to prevent non-specific binding, washing the ELISA plate, adding a solution containing the albumin fusion protein of the invention (at one or more different concentrations), adding a secondary anti-Therapeutic protein specific antibody coupled to a detectable label (as described herein or otherwise known in the art), and detecting the presence of the secondary antibody. In an alternate version of this protocol, the ELISA plate might be coated with the anti-Therapeutic protein specific antibody and the labeled secondary reagent might be the anti-human albumin specific antibody.

Uses of the Polynucleotides

[0369] Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

[0370] The polynucleotides of the present invention are useful to produce the albumin fusion proteins of the invention. As described in more detail below, polynucleotides of the invention (encoding albumin fusion proteins) may be used in recombinant DNA methods useful in genetic engineering to make cells, cell lines, or tissues that express the albumin fusion protein encoded by the polynucleotides encoding albumin fusion proteins of the invention.

[0371] Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell. Additional non-limiting examples of gene therapy methods encompassed by the present invention are more thoroughly described elsewhere herein (see, e.g., the sections labeled "Gene Therapy", and Examples 61 and 62).

Uses of the Polypeptides

[0372] Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

[0373] Albumin fusion proteins of the invention are useful to provide immunological probes for differential identification of the tissue(s) (e.g., immunohistochemistry assays such as, for example, ABC immunoperoxidase (Hsu et al., J. Histochem. Cytochem. 29:577-580 (1981)) or cell type(s) (e.g., immunocytochemistry assays).

[0374] Albumin fusion proteins can be used to assay levels of polypeptides in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell. Biol. 105:3087-3096 (1987)). Other methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (^{131}I, ^{125}I, ^{123}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115m}In, ^{113m}In, ^{112}In, ^{111}In), and technetium (^{99}Tc, ^{99m}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0375] Albumin fusion proteins of the invention can also be detected *in vivo* by imaging. Labels or markers for *in vivo* imaging of protein include those detectable by X-radiography, nuclear magnetic resonance (NMR) or electron spin relaxation (ESR). For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the albumin fusion protein by labeling of nutrients given to a cell line expressing the albumin fusion protein of the invention.

[0376] An albumin fusion protein which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I, ^{112}In, ^{99m}Tc, (^{131}I, ^{125}I, ^{123}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115m}In, ^{113m}In, ^{112}In, ^{111}In), and technetium (^{99}Tc, ^{99m}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F, ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for

example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for immune system disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled albumin fusion protein will then preferentially accumulate at locations in the body (e.g., organs, cells, extracellular spaces or matrices) where one or more receptors, ligands or substrates (corresponding to that of the Therapeutic protein used to make the albumin fusion protein of the invention) are located. Alternatively, in the case where the albumin fusion protein comprises at least a fragment or variant of a Therapeutic antibody, the labeled albumin fusion protein will then preferentially accumulate at the locations in the body (e.g., organs, cells, extracellular spaces or matrices) where the polypeptides/epitopes corresponding to those bound by the Therapeutic antibody (used to make the albumin fusion protein of the invention) are located. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)). The protocols described therein could easily be modified by one of skill in the art for use with the albumin fusion proteins of the invention.

[0377] In one embodiment, the invention provides a method for the specific delivery of albumin fusion proteins of the invention to cells by administering albumin fusion proteins of the invention (e.g., polypeptides encoded by polynucleotides encoding albumin fusion proteins of the invention and/or antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a Therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

[0378] In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering albumin fusion proteins of the invention in association with toxins or cytotoxic prodrugs.

[0379] By "toxin" is meant one or more compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNAse, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. "Toxin" also includes a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi, or other radioisotopes such as, for example, ^{103}Pd, ^{133}Xe, ^{131}I, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{35}S, ^{90}Y, ^{153}Sm, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{113}Sn, ^{90}Yttrium, ^{117}Tin, ^{186}Rhenium, ^{166}Holmium, and ^{188}Rhenium; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin. In a specific embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention or antibodies of the invention in association with the radioisotope ^{90}Y. In another specific embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention or antibodies of the invention in association with the radioisotope ^{111}In. In a further specific embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention or antibodies of the invention in association with the radioisotope ^{131}I.

[0380] Techniques known in the art may be applied to labe polypeptides of the invention. Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274;119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety).

[0381] The albumin fusion proteins of the present invention are useful for diagnosis, treatment, prevention and/or prognosis of various disorders in mammals, preferably humans. Such disorders include, but are not limited to, those described herein under the section heading "Biological Activities," below.

[0382] Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression level of a certain polypeptide in cells or body fluid of an individual using an albumin fusion protein of the invention; and (b) comparing the assayed polypeptide expression level with a standard polypeptide expression level, whereby an increase or decrease in the assayed polypeptide expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

[0383] Moreover, albumin fusion proteins of the present invention can be used to treat or prevent diseases or conditions such as, for example, neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement

absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor supressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the immune response to proliferative cells or tissues).

[0384] In particular, albumin fusion proteins comprising of at least a fragment or variant of a Therapeutic antibody can also be used to treat disease (as described *supra*, and elsewhere herein). For example, administration of an albumin fusion protein comprising of at least a fragment or variant of a Therapeutic antibody can bind, and/or neutralize the polypeptide to which the Therapeutic antibody used to make the albumin fusion protein specifically binds, and/or reduce overproduction of the polypeptide to which the Therapeutic antibody used to make the albumin fusion protein specifically binds. Similarly, administration of an albumin fusion protein comprising of at least a fragment or variant of a Therapeutic antibody can activate the polypeptide to which the Therapeutic antibody used to make the albumin fusion protein specifically binds, by binding to the polypeptide bound to a membrane (receptor).

[0385] At the very least, the albumin fusion proteins of the invention of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Albumin fusion proteins of the invention can also be used to raise antibodies, which in turn may be used to measure protein expression of the Therapeutic protein, albumin protein, and/or the albumin fusion protein of the invention from a recombinant cell, as a way of assessing transformation of the host cell, or in a biological sample. Moreover, the albumin fusion proteins of the present invention can be used to test the biological activities described herein.

Diagnostic Assays

[0386] The compounds of the present invention are useful for diagnosis, treatment, prevention and/or prognosis of various disorders in mammals, preferably humans. Such disorders include, but are not limited to, those described for each Therapeutic protein in the corresponding row of Table 1 and herein under the section headings "Immune Activity," "Blood Related Disorders," "Hyperproliferative Disorders," "Renal Disorders," "Cardiovascular Disorders," "Respiratory Disorders," "Anti-Angiogenesis Activity," "Diseases at the Cellular Level," "Wound Healing and Epithelial Cell Proliferation," "Neural Activity and Neurological Diseases," "Endocrine Disorders," "Reproductive System Disorders," "Infectious Disease," "Regeneration," and/or "Gastrointestinal Disorders," *infra*.

[0387] For a number of disorders, substantially altered (increased or decreased) levels of gene expression can be detected in tissues, cells or bodily fluids (e.g., sera, plasma, urine, semen, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" gene expression level, that is, the expression level in tissues or bodily fluids from an individual not having the disorder. Thus, the invention provides a diagnostic method useful during diagnosis of a disorder, which involves measuring the expression level of the gene encoding a polypeptide in tissues, cells or body fluid from an individual and comparing the measured gene expression level with a standard gene expression level, whereby an increase or decrease in the gene expression level(s) compared to the standard is indicative of a disorder. These diagnostic assays may be performed *in vivo* or *in vitro*, such as, for example, on blood samples, biopsy tissue or autopsy tissue.

[0388] The present invention is also useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed gene expression will experience a worse clinical outcome.

[0389] By "assaying the expression level of the gene encoding a polypeptide" is intended qualitatively or quantitatively measuring or estimating the level of a particular polypeptide (e.g. a polypeptide corresponding to a Therapeutic protein disclosed in Table 1) or the level of the mRNA encoding the polypeptide of the invention in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide expression level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having the disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

[0390] By "biological sample" is intended any biological sample obtained from an individual, cell line, tissue culture, or other source containing polypeptides of the invention (including portions thereof) or mRNA. As indicated, biological samples include body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) and tissue sources found to express the full length or fragments thereof of a polypeptide or mRNA. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

[0391] Total cellular RNA can be isolated from a biological sample using any suitable technique such as the single-step guanidinium-thiocyanate-phenol-chloroform method described in Chomczynski and Sacchi, Anal. Biochem. 162:156-159 (1987). Levels of mRNA encoding the polypeptides of the invention are then assayed using any appropriate method. These include Northern blot analysis, S1 nuclease mapping, the polymerase chain reaction (PCR), reverse transcription in combination with the polymerase chain reaction (RT-PCR), and reverse transcription in combination with the ligase chain reaction (RT-LCR).

[0392] The present invention also relates to diagnostic assays such as quantitative and diagnostic assays for detecting levels of polypeptides that

bind to, are bound by, or associate with albumin fusion proteins of the invention, in a biological sample (e.g., cells and tissues), including determination of normal and abnormal levels of polypeptides. Thus, for instance, a diagnostic assay in accordance with the invention for detecting abnormal expression of polypeptides that bind to, are bound by, or associate with albumin fusion proteins compared to normal control tissue samples may be used to detect the presence of tumors. Assay techniques that can be used to determine levels of a polypeptide that bind to, are bound by, or associate with albumin fusion proteins of the present invention in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays. Assaying polypeptide levels in a biological sample can occur using any art-known method.

[0393] Assaying polypeptide levels in a biological sample can occur using a variety of techniques. For example, polypeptide expression in tissues can be studied with classical immunohistological methods (Jalkanen et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell . Biol. 105:3087-3096 (1987)). Other methods useful for detecting polypeptide gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium (^{99m}Tc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0394] The tissue or cell type to be analyzed will generally include those which are known, or suspected, to express the gene of interest (such as, for example, cancer). The protein isolation methods employed herein may, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), which is incorporated herein by reference in its entirety. The isolated cells can be derived from cell culture or from a patient. The analysis of cells taken from culture may be a necessary step in the assessment of cells that could be used as part of a cell-based gene therapy technique or, alternatively, to test the effect of compounds on the expression of the gene.

[0395] For example, albumin fusion proteins may be used to quantitatively or qualitatively detect the presence of polypeptides that bind to, are bound by, or associate with albumin fusion proteins of the present invention. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled albumin fusion protein coupled with light microscopic, flow cytometric, or fluorimetric detection.

[0396] In a preferred embodiment, albumin fusion proteins comprising at least a fragment or variant of an antibody that specifically binds at least a Therapeutic protein disclosed herein (e.g., the Therapeutic proteins disclosed in Table 1) or otherwise known in the art may be used to quantitatively or qualitatively detect the presence of gene products or conserved variants or peptide fragments thereof. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric, or fluorimetric detection.

[0397] The albumin fusion proteins of the present invention may, additionally, be employed histologically, as in immunofluorescence, immunoelectron microscopy or non-immunological assays, for in situ detection of polypeptides that bind to, are bound by, or associate with an albumin fusion protein of the present invention. In situ detection may be accomplished by removing a histological specimen from a patient, and applying thereto a labeled antibody or polypeptide of the present invention. The albumin fusion proteins are preferably applied by overlaying the labeled albumin fusion protein onto a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the polypeptides that bind to, are bound by, or associate with albumin fusion proteins, but also its distribution in the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such in situ detection.

[0398] Immunoassays and non-immunoassays that detect polypeptides that bind to, are bound by, or associate with albumin fusion proteins will typically comprise incubating a sample, such as a biological fluid, a tissue extract, freshly harvested cells, or lysates of cells which have been incubated in cell culture, in the presence of a detectably labeled antibody capable of binding gene products or conserved variants or peptide fragments thereof, and detecting the bound antibody by any of a number of techniques well-known in the art.

[0399] The biological sample may be brought in contact with and immobilized onto a solid phase support or carrier such as nitrocellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled albumin fusion protein of the invention. The solid phase support may then be washed with the buffer a second time to remove unbound antibody or polypeptide. Optionally the antibody is subsequently labeled. The amount of bound label on solid support may then be detected by conventional means.

[0400] By "solid phase support or carrier" is intended any support capable of binding a polypeptide (e.g., an albumin fusion protein, or polypeptide that binds, is bound by, or associates with an albumin fusion protein of the invention.) Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to a polypeptide. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other

suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

[0401] The binding activity of a given lot of albumin fusion protein may be determined according to well known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

[0402] In addition to assaying polypeptide levels in a biological sample obtained from an individual, polypeptide can also be detected *in vivo* by imaging. For example, in one embodiment of the invention, albumin fusion proteins of the invention are used to image diseased or neoplastic cells.

[0403] Labels or markers for *in vivo* imaging of albumin fusion proteins of the invention include those detectable by X-radiography, NMR, MRI, CAT-scans or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the albumin fusion protein by labeling of nutrients of a cell line (or bacterial or yeast strain) engineered.

[0404] Additionally, albumin fusion proteins of the invention whose presence can be detected, can be administered. For example, albumin fusion proteins of the invention labeled with a radio-opaque or other appropriate compound can be administered and visualized *in vivo*, as discussed, above for labeled antibodies. Further, such polypeptides can be utilized for *in vitro* diagnostic procedures.

[0405] A polypeptide-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I, ^{112}In, ^{99m}Tc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for a disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled albumin fusion protein will then preferentially accumulate at the locations in the body which contain a polypeptide or other substance that binds to, is bound by or associates with an albumin fusion protein of the present invention. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

[0406] One of the ways in which an albumin fusion protein of the present invention can be detectably labeled is by linking the same to a reporter enzyme and using the linked product in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent Assay (ELISA)", 1978, Diagnostic Horizons 2:1-7, Microbiological Associates Quarterly Publication, Walkersville, MD); Voller et al., *J. Clin. Pathol.* 31:507-520 (1978); Butler, J.E., *Meth. Enzymol.* 73:482-523 (1981); Maggio, E. (ed.), 1980, Enzyme Immunoassay, CRC Press, Boca Raton, FL.; Ishikawa, E. et al., (eds.), 1981, Enzyme Immunoassay, Kgaku Shoin, Tokyo). The reporter enzyme which is bound to the antibody will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorimetric or by visual means. Reporter enzymes which can be used to detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. Additionally, the detection can be accomplished by colorimetric methods which employ a chromogenic substrate for the reporter enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

[0407] Albumin fusion proteins may also be radiolabelled and used in any of a variety of other immunoassays. For example, by radioactively labeling the albumin fusion proteins, it is possible to the use the albumin fusion proteins in a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by means including, but not limited to, a gamma counter, a scintillation counter, or autoradiography.

[0408] Additionally, chelator molecules, are known in the art and can be used to label the Albumin fusion proteins. Chelator molecules may be attached Albumin fusion proteins of the invention to facilitate labeling said protein with metal ions including radionuclides or fluorescent labels. For example, see Subramanian, R. and Meares, C.F., "Bifunctional Chelating Agents for Radiometal-labeled monoclonal Antibodies," in *Cancer Imaging with Radiolabeled Antibodies* (D. M. Goldenberg, Ed.) Kluwer Academic Publications, Boston; Saji, H., "Targeted delivery of radiolabeled imaging and therapeutic agents: bifunctional radiopharmaceuticals." *Crit. Rev. Ther. Drug Carrier Syst. 16*:209-244 (1999); Srivastava S.C. and Mease R.C., "Progress in research on ligands, nuclides and techniques for labeling monoclonal antibodies." *Int. J. Rad. Appl. Instrum. B 18*:589-603 (1991); and Liu, S. and Edwards, D.S., "Bifunctional chelators for therapeutic lanthanide radiopharmaceuticals." *Bioconjug. Chem. 12*:7-34 (2001). Any chelator which can be covalently bound to said Albumin fusion proteins may be used according to the present invention. The chelator may further comprise a linker moiety that connects the chelating moiety to the Albumin fusion protein.

[0409] In one embodiment, the Albumin fusion protein of the invention are attached to an acyclic chelator such as diethylene triamine-N,N,N',N",N"-pentaacetic acid (DPTA), analogues of DPTA, and derivatives of DPTA. As non-limiting examples, the chelator may be 2-(p-isothiocyanatobenzyl)-6- methyldiethylenetriaminepentaacetic acid (1B4M-DPTA, also known as MX-DTPA), 2-methyl-6-(rho-nitrobenzyl)-1,4,7-triazaheptane-N,N,N',N",N"-pentaacetic acid (nitro-1B4M-DTPA or nitro-MX-DTPA); 2-(p-isothiocyanatobenzyl)-

cyclohexyldiethylenetriaminepentaacetic acid (CHX-DTPA), or N-[2-amino-3-(rho-nitrophenyl)propyl]-trans-cyclohexane-1,2-diamine-N,N',N"-pentaacetic acid (nitro-CHX-A-DTPA).

[0410] In another embodiment, the Albumin fusion protein of the invention are attached to an acyclic terpyridine chelator such as 6,6"-bis[[N,N,N",N"- tetra(carboxymethyl)amino]methyl]-4'-(3-amino-4-methoxyphenyl)-2,2':6',2 "- terpyridine (TMT-amine).

[0411] In specific embodiments, the macrocyclic chelator which is attached to the the Albumin fusion protein of the invention is 1,4,7,10-tetraazacyclododecane-N,N',N",N"'-tetraacetic acid (DOTA). In other specific embodiments, the DOTA is attached to the the Albumin fusion protein of the invention via a linker molecule. Examples of linker molecules useful for conjugating DOTA to a polypeptide are commonly known in the art - see, for example, DeNardo *et al., Clin. Cancer Res.* 4(10):2483-90, 1998; Peterson *et al., Bioconjug. Chem.* 10(4):553-7, 1999; and Zimmerman *et al., Nucl. Med. Biol.* 26(8):943-50, 1999 which are hereby incorporated by reference in their entirety. In addition, U.S. Patents 5,652,361 and 5,756,065, which disclose chelating agents that may be conjugated to antibodies, and methods for making and using them, are hereby incorporated by reference in their entireties. Though U.S. Patents 5,652,361 and 5,756,065 focus on conjugating chelating agents to antibodies, one skilled in the art could readily adapt the method disclosed therein in order to conjugate chelating agents to other polypeptides.

[0412] Bifunctional chelators based on macrocyclic ligands in which conjugation is via an activated arm, or functional group, attached to the carbon backbone of the ligand can be employed as described by M. Moi *et al., J. Amer. Chem. Soc.* 49:2639 (1989) (2-*p*-nitrobenzyl-1,4,7,10-tetraazacyclododecane-N,N',N",N"'-tetraacetic acid); S. V. Deshpande *et al., J. Nucl. Med.* 31:473 (1990); G. Ruser *et al., Bioconj. Chem.* 1:345 (1990); C. J. Broan *et al., J. C. S. Chem. Comm.* 23:1739 (1990); and C. J. Anderson *et al., J. Nucl. Med.* 36:850 (1995).

[0413] In one embodiment, a macrocyclic chelator, such as polyazamacrocyclic chelators, optionally containing one or more carboxy, amino, hydroxamate, phosphonate, or phosphate groups, are attached to the Albumin fusion protein of the invention. In another embodiment, the chelator is a chelator selected from the group consisting of DOTA, analogues of DOTA, and derivatives of DOTA.

[0414] In one embodiment, suitable chelator molecules that may be attached to the the Albumin fusion protein of the invention include DOXA (1-oxa-4,7,10-triazacyclododecanetriacetic acid), NOTA (1,4,7-triazacyclononanetriacetic acid), TETA (1,4,8,11-tetraazacyclotetradecanetetraacetic acid), and THT (4'-(3-amino-4-methoxy-phenyl)-6,6"-bis(N',N'-dicarboxymethyl-*N*-methylhydrazino)-2,2':6',2"-terpyridine), and analogs and derivatives thereof. See, e.g., Otmono *et al., J. Med. Chem.* 35: 157-162 (1992); Kung *et al., J. Nucl. Med.* 25: 326-332 (1984); Jurisson *et al., Chem. Rev.* 93:1137-1156 (1993); and U.S. Patent No. 5,367,080. Other suitable chelators include chelating agents disclosed in U.S. Patent Nos. 4,647,447; 4,687,659; 4,885,363; EP-A-71564; WO89/00557; and EP-A-232751.

[0415] In another embodiment, suitable macrocyclic carboxylic acid chelators which can be used in the present invention include 1,4,7,10-tetraazacyclododecane-*N,N',N",N"'*-tetraacetic acid (DOTA); 1,4,8,12-tetraazacyclopentadecane-*N,N',N",N"'*-tetraacetic acid (15N4); 1,4,7-triazacyclononane-N,N',N"-triacetic acid (9N3); 1,5,9-triazacyclododecane-N,N',N"-triacetic acid (12N3); and 6-bromoacetamido-benzyl-1,4,8,11-tetraazacyclotetradecane- *N,N',N",N"'*-tetraacetic acid (BAT).

[0416] A preferred chelator that can be attached to the Albumin Fusion protein of the invention is α-(5-isothiocyanato- 2-methoxyphenyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, which is also known as MeO-DOTA-NCS. A salt or ester of α-(5-isothiocyanato- 2-methoxyphenyl)- 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid may also be used.

[0417] Albumin fusion proteins of the invention to which chelators such as those decribed are covalently attached may be labeled (via the coordination site of the chelator) with radionuclides that are suitable for therapeutic, diagnostic, or both therapeutic and diagnostic purposes. Examples of appropriate metals include Ag, At, Au, Bi, Cu, Ga, Ho, In, Lu, Pb, Pd, Pm, Pr, Rb, Re, Rh, Sc, Sr, Tc, Tl, Y, and Yb. Examples of the radionuclide used for diagnostic purposes are Fe, Gd, ^{111}In, ^{67}Ga, or ^{68}Ga. In another embodiment, the radionuclide used for diagnostic purposes is ^{111}In, or ^{67}Ga. Examples of the radionuclide used for therapeutic purposes are ^{166}Ho, ^{165}Dy, ^{90}Y, ^{115m}In, ^{55}Fe, or ^{72}Ga. In one embodiment, the radionuclide used for diagnostic purposes is ^{166}Ho or ^{90}Y. Examples of the radionuclides used for both therapeutic and diagnostic purposes include ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{169}Yb, or ^{47}Sc. In one embodiment, the radionuclide is ^{153}Sm, ^{177}Lu, ^{169}Yb, or ^{159}Gd.

[0418] Preferred metal radionuclides include ^{90}Y, ^{99m}Tc, ^{111}In, ^{47}Sc, ^{67}Ga, ^{51}Cr, ^{177m}Sn, ^{67}Cu, ^{167}Tm, ^{97}Ru, ^{188}Re, ^{177}Lu, ^{199}Au, ^{47}Sc, ^{67}Ga, ^{51}Cr, ^{117m}Sn, ^{67}Cu, ^{167}Tm, ^{97}Ru, ^{188}Re, ^{177}Lu, ^{199}Au, ^{203}Pb and ^{141}Ce.

[0419] In a particular embodiment, Albumin fusion proteins of the invention to which chelators are covalently attached may be labeled with a metal ion selected from the group consisting of ^{90}Y, ^{111}In, ^{177}Lu, ^{166}Ho, ^{213}Bi, and ^{225}Ac.

[0420] Moreover, γ-emitting radionuclides, such as ^{99m}Tc, ^{111}In, ^{67}Ga, and ^{169}Yb have been approved or under investigation for diagnostic imaging, while β-emitters, such as ^{67}Cu, ^{111}Ag, ^{186}Re, and ^{90}Y are useful for the applications in tumor therapy. Also other useful radionuclides include γ-emitters, such as ^{99m}Tc, ^{111}In, ^{67}Ga, and ^{169}Yb, and β-emitters, such as ^{67}Cu, ^{111}Ag, ^{186}Re, ^{188}Re and ^{90}Y, as well as other radionuclides of interest such as ^{211}At, ^{212}Bi, ^{177}Lu, ^{86}Rb , ^{105}Rh, ^{153}Sm, ^{198}Au, ^{149}Pm, ^{85}Sr, ^{142}Pr, ^{214}Pb, ^{109}Pd, ^{166}Ho, ^{203}Tl, and ^{44}Sc. Albumin fusion proteins of the invention to which chelators are covalently attached may be labeled with the radionuclides described above.

[0421] In another embodiment, Albumin fusion proteins of the invention to which chelators are covalently attached may be labeled with paramagnetic metal ions including ions of transition and lanthanide metal, such as metals having atomic numbers of 21-29, 42, 43, 44, or 57-71, in particular ions of Cr, V, Mn, Fe, Co, Ni, Cu, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu. The paramagnetic metals used in

compositions for magnetic resonance imaging include the elements having atomic numbers of 22 to 29, 42, 44 and 58-70.

[0422] In another embodiment, Albumin fusion proteins of the invention to which chelators are covalently attached may be labeled with fluorescent metal ions including lanthanides, in particular La, Ce, Pr, Nd, Pm, Sm, Eu (e.g., ^{152}Eu), Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu.

[0423] In another embodiment, Albumin fusion proteins of the invention to which chelators are covalently attached may be labeled with heavy metal-containing reporters may include atoms of Mn, Bi, Si, and W.

[0424] It is also possible to label the albumin fusion proteins with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, ophthaldehyde and fluorescamine.

[0425] The albumin fusion protein can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

[0426] The albumin fusion proteins can also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged albumin fusion protein is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

[0427] Likewise, a bioluminescent compound may be used to label albumin fusion proteins of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

Transgenic Organisms

[0428] Transgenic organisms that express the albumin fusion proteins of the invention are also included in the invention. Transgenic organisms are genetically modified organisms into which recombinant, exogenous or cloned genetic material has been transferred. Such genetic material is often referred to as a transgene. The nucleic acid sequence of the transgene may include one or more transcriptional regulatory sequences and other nucleic acid sequences such as introns, that may be necessary for optimal expression and secretion of the encoded protein. The transgene may be designed to direct the expression of the encoded protein in a manner that facilitates its recovery from the organism or from a product produced by the organism, e.g. from the milk, blood, urine, eggs, hair or seeds of the organism. The transgene may consist of nucleic acid sequences derived from the genome of the same species or of a different species than the species of the target animal. The transgene may be integrated either at a locus of a genome where that particular nucleic acid sequence is not otherwise normally found or at the normal locus for the transgene.

[0429] The term "germ cell line transgenic organism" refers to a transgenic organism in which the genetic alteration or genetic information was introduced into a germ line cell, thereby conferring the ability of the transgenic organism to transfer the genetic information to offspring. If such offspring in fact possess some or all of that alteration or genetic information, then they too are transgenic organisms. The alteration or genetic information may be foreign to the species of organism to which the recipient belongs, foreign only to the particular individual recipient, or may be genetic information already possessed by the recipient. In the last case, the altered or introduced gene may be expressed differently than the native gene.

[0430] A transgenic organism may be a transgenic animal or a transgenic plant. Transgenic animals can be produced by a variety of different methods including transfection, electroporation, microinjection, gene targeting in embryonic stem cells and recombinant viral and retroviral infection (*see, e.g.*, U.S. Patent No. 4,736,866; U.S. Patent No. 5,602,307; Mullins *et al.* (1993) Hypertension 22(4):630-633; Brenin *et al.* (1997) Surg. Oncol. 6(2)99-110; Tuan (ed.), *Recombinant Gene Expression Protocols*, Methods in Molecular Biology No. 62, Humana Press (1997)). The method of introduction of nucleic acid fragments into recombination competent mammalian cells can be by any method which favors co-transformation of multiple nucleic acid molecules. Detailed procedures for producing transgenic animals are readily available to one skilled in the art, including the disclosures in U.S. Patent No. 5,489,743 and U.S. Patent No. 5,602,307.

[0431] A number of recombinant or transgenic mice have been produced, including those which express an activated oncogene sequence (U.S. Patent No. 4,736,866); express simian SV40 T-antigen (U.S. Patent No. 5,728,915); lack the expression of interferon regulatory factor 1 (IRF-1) (U.S. Patent No. 5,731,490); exhibit dopaminergic dysfunction (U.S. Patent No. 5,723,719); express at least one human gene which participates in blood pressure control (U.S. Patent No. 5,731,489); display greater similarity to the conditions existing in naturally occurring Alzheimer's disease (U.S. Patent No. 5,720,936); have a reduced capacity to mediate cellular adhesion (U.S. Patent No. 5,602,307); possess a bovine growth hormone gene (Clutter *et al.* (1996) Genetics 143(4):1753-1760); or, are capable of generating a fully human antibody response (McCarthy (1997) The Lancet 349(9049):405).

[0432] While mice and rats remain the animals of choice for most transgenic experimentation, in some instances it is preferable or even necessary to use alternative animal species. Transgenic procedures have been successfully utilized in a variety of non-murine animals, including sheep, goats, pigs, dogs, cats, monkeys, chimpanzees, hamsters, rabbits, cows and guinea pigs (*see, e.g.*, Kim *et al.* (1997) Mol. Reprod. Dev.

46(4):515-526; Houdebine (1995) Reprod. Nutr. Dev. 35(6):609-617; Petters (1994) Reprod. Fertil. Dev. 6(5):643-645; Schnieke *et al.* (1997) Science 278(5346):2130-2133; and Amoah (1997) J. Animal Science 75(2):578-585).

[0433] To direct the secretion of the transgene-encoded protein of the invention into the milk of transgenic mammals, it may be put under the control of a promoter that is preferentially activated in mammary epithelial cells. Promoters that control the genes encoding milk proteins are preferred, for example the promoter for casein, beta lactoglobulin, whey acid protein, or lactalbumin (see, *e.g.*, DiTullio (1992) BioTechnology 10:74-77; Clark *et al.* (1989) BioTechnology 7:487-492; Gorton *et al.* (1987) BioTechnology 5:1183-1187; and Soulier *et al.* (1992) FEBS Letts. 297:13). The transgenic mammals of choice would produce large volumes of milk and have long lactating periods, for example goats, cows, camels or sheep.

[0434] An albumin fusion protein of the invention can also be expressed in a transgenic plant, e.g. a plant in which the DNA transgene is inserted into the nuclear or plastidic genome. Plant transformation procedures used to introduce foreign nucleic acids into plant cells or protoplasts are known in the art. See, in general, Methods in Enzymology Vol. 153 ("Recombinant DNA Part D") 1987, Wu and Grossman Eds., Academic Press and European Patent Application EP 693554. Methods for generation of genetically engineered plants are further described in US Patent No. 5,283,184, US Patent No. 5, 482,852, and European Patent Application EP 693 554, all of which are hereby incorporated by reference.

Pharmaceutical or Therapeutic Compositions

[0435] The albumin fusion proteins of the invention or formulations thereof may be administered by any conventional method including parenteral (*e.g.* subcutaneous or intramuscular) injection or intravenous infusion. The treatment may consist of a single dose or a plurality of doses over a period of time.

[0436] While it is possible for an albumin fusion protein of the invention to be administered alone, it is preferable to present it as a pharmaceutical formulation, together with one or more acceptable carriers. The carrier(s) must be "acceptable" in the sense of being compatible with the albumin fusion protein and not deleterious to the recipients thereof. Typically, the carriers will be water or saline which will be sterile and pyrogen free. Albumin fusion proteins of the invention are particularly well suited to formulation in aqueous carriers such as sterile pyrogen free water, saline or other isotonic solutions because of their extended shelf-life in solution. For instance, pharmaceutical compositions of the invention may be formulated well in advance in aqueous form, for instance, weeks or months or longer time periods before being dispensed.

[0437] For example, formulations containing the albumin fusion protein may be prepared taking into account the extended shelf-life of the albumin fusion protein in aqueous formulations. As discussed above, the shelf-life of many of these Therapeutic proteins are markedly increased or prolonged after fusion to HA.

[0438] In instances where aerosol administration is appropriate, the albumin fusion proteins of the invention can be formulated as aerosols using standard procedures. The term "aerosol" includes any gas-borne suspended phase of an albumin fusion protein of the instant invention which is capable of being inhaled into the bronchioles or nasal passages. Specifically, aerosol includes a gas-borne suspension of droplets of an albumin fusion protein of the instant invention, as may be produced in a metered dose inhaler or nebulizer, or in a mist sprayer. Aerosol also includes a dry powder composition of a compound of the instant invention suspended in air or other carrier gas, which may be delivered by insufflation from an inhaler device, for example. See Ganderton & Jones, *Drug Delivery to the Respiratory Tract, Ellis Horwood* (19 87); Gonda (1990) *Critical Reviews in Therapeutic Drug Carrier Systems* 6:273-313; and Raeburn *et al.*. (1992) *Pharmacol. Toxicol. Methods* 27:143-159.

[0439] The formulations of the invention are also typically non-immunogenic, in part, because of the use of the components of the albumin fusion protein being derived from the proper species. For instance, for human use, both the Therapeutic protein and albumin portions of the albumin fusion protein will typically be human. In some cases, wherein either component is non human-derived, that component may be humanized by substitution of key amino acids so that specific epitopes appear to the human immune system to be human in nature rather than foreign.

[0440] The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the albumin fusion protein with the carrier that constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

[0441] Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation appropriate for the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampules, vials or syringes, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders. Dosage formulations may contain the Therapeutic protein portion at a lower molar concentration or lower dosage compared to the non-fused standard formulation for the Therapeutic protein given the extended serum half-life exhibited by many of the albumin fusion proteins of the invention.

[0442] As an example, when an albumin fusion protein of the invention comprises one of the proteins listed in the "Therapeutic Protein:X" column of Table 1 as one or more of the Therapeutic protein regions, the dosage form can be calculated on the basis of the potency of the albumin

fusion protein relative to the potency of the therapeutic protein alone, while taking into account the prolonged serum half-life and shelf-life of the albumin fusion proteins compared to that of native therapeutic protein. For example, if the therapeutic protein is typically administered at 0.3 to 30.0 IU/kg/week, or 0.9 to 12.0 IU/kg/week, given in three or seven divided doses for a year or more. In an albumin fusion protein consisting of full length HA fused to a therpeutic protein, an equivalent dose in terms of units would represent a greater weight of agent but the dosage frequency can be reduced, for example to twice a week, once a week or less.

[0443] Formulations or compositions of the invention may be packaged together with, or included in a kit with, instructions or a package insert referring to the extended shelf-life of the albumin fusion protein component. For instance, such instructions or package inserts may address recommended storage conditions, such as time, temperature and light, taking into account the extended or prolonged shelf-life of the albumin fusion proteins of the invention. Such instructions or package inserts may also address the particular advantages of the albumin fusion proteins of the inventions, such as the ease of storage for formulations that may require use in the field, outside of controlled hospital, clinic or office conditions. As described above, formulations of the invention may be in aqueous form and may be stored under less than ideal circumstances without significant loss of therapeutic activity.

[0444] Albumin fusion proteins of the invention can also be included in nutraceuticals. For instance, certain albumin fusion proteins of the invention may be administered in natural products, including milk or milk product obtained from a transgenic mammal which expresses albumin fusion protein. Such compositions can also include plant or plant products obtained from a transgenic plant which expresses the albumin fusion protein. The albumin fusion protein can also be provided in powder or tablet form, with or without other known additives, carriers, fillers and diluents. Nutraceuticals are described in Scott Hegenhart, *Food Product Design*, Dec. 1993.

[0445] The invention also provides methods of treatment and/or prevention of diseases or disorders (such as, for example, any one or more of the diseases or disorders disclosed herein) by administration to a subject of an effective amount of an albumin fusion protein of the invention or a polynucleotide encoding an albumin fusion protein of the invention ("albumin fusion polynucleotide") in a pharmaceutically acceptable carrier.

[0446] The albumin fusion protein and/or polynucleotide will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the albumin fusion protein and/or polynucleotide alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

[0447] As a general proposition, the total pharmaceutically effective amount of the albumin fusion protein administered parenterally per dose will be in the range of about 1ug/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the albumin fusion protein is typically administered at a dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

[0448] As noted above, the albumin fusion protein of the invention has a higher plasma stability compared to the Therapeutic protein portion (or fragment or variant thereof) alone. This increase in plasma stability should be taken into account when determining the effective amount of the albumin fusion protein to be administered per dose and the dosing administration schedule. In particular, higher plasma stability may allow the albumin fusion protein to be administered at a lower dose at the same frequency of administrations, or alternatively, may allow the albumin fusion protein to be administered in fewer dosings. Preferably, the higher stability allows the albumin fusion protein of the invention to be administered less often in fewer dosings. More preferably, the albumin fusion protein can be administered once every two weeks. Still more preferably, the albumin fusion protein can be administered once every three, four, five, or more weeks depending on the pharmacokinetics of the albumin fusion protein. For example, as discussed above, the pharmacokinetics of an IFN-alpha-HSA fusion protein supports a dosing regimen of once every 2-4 weeks or more, and even dosing at intervals of 4 weeks or more than every 4 weeks.

[0449] Albumin fusion proteins and/or polynucleotides can be are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), bucally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

[0450] Albumin fusion proteins and/or polynucleotides of the invention are also suitably administered by sustained-release systems. Examples of sustained-release albumin fusion proteins and/or polynucleotides are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), bucally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion. Additional examples of sustained-release albumin fusion proteins and/or polynucleotides

include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

[0451] Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., Biopolymers 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (Langer et al., J. Biomed. Mater. Res. 15:167-277 (1981), and Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., id.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988).

[0452] Sustained-release albumin fusion proteins and/or polynucleotides also include liposomally entrapped albumin fusion proteins and/or polynucleotides of the invention (*see* generally, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing the albumin fusion protein and/or polynucleotide are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci.(USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Therapeutic.

[0453] In yet an additional embodiment, the albumin fusion proteins and/or polynucleotides of the invention are delivered by way of a pump (*see* Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)).

[0454] Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

[0455] For parenteral administration, in one embodiment, the albumin fusion protein and/or polynucleotide is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

[0456] Generally, the formulations are prepared by contacting the albumin fusion protein and/or polynucleotide uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

[0457] The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, *and* include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates (including, for example, Tween-20), poloxamers, or PEG.

[0458] The albumin fusion protein is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

[0459] Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Albumin fusion proteins and/or polynucleotides generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0460] Albumin fusion proteins and/or polynucleotides ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous albumin fusion protein and/or polynucleotide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized albumin fusion protein and/or polynucleotide using bacteriostatic Water-for-Injection.

[0461] In a specific and preferred embodiment, the Albumin fusion protein formulations comprises 0.01 M sodium phosphate, 0.15 mM sodium chloride, 0.16 micromole sodium octanoate/milligram of fusion protein, 15 micrograms/milliliter polysorbate 80, pH 7.2. In another specific and preferred embodiment, the Albumin fusion protein formulations consists 0.01 M sodium phosphate, 0.15 mM sodium chloride, 0.16 micromole sodium octanoate/milligram of fusion protein, 15 micrograms/milliliter polysorbate 80, pH 7.2. The pH and buffer are chosen to match physiological conditions and the salt is added as a tonicifier. Sodium octanoate has been chosen due to its reported ability to increase the thermal stability of the protein in solution. Finally, polysorbate has been added as a generic surfactant, which lowers the surface tension of the solution and

lowers non-specific adsorption of the albumin fusion protein to the container closure system.

[0462] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the albumin fusion proteins and/or polynucleotides of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the albumin fusion proteins and/or polynucleotides may be employed in conjunction with other therapeutic compounds.

[0463] The albumin fusion proteins and/or polynucleotides of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG (e.g., THERACYS®), MPL and nonviable preparations of *Corynebacterium parvum*. In a specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are administered in combination with alum. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diptheria, hepatitis A, hepatitis B, *Haemophilus influenzae* B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

[0464] The albumin fusion proteins and/or polynucleotides of the invention may be administered alone or in combination with other therapeutic agents. Albumin fusion protein and/or polynucleotide agents that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention, include but not limited to, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, and/or therapeutic treatments described below. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

[0465] In one embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with an anticoagulant. Anticoagulants that may be administered with the compositions of the invention include, but are not limited to, heparin, low molecular weight heparin, warfarin sodium (e.g., COUMADIN®), dicumarol, 4-hydroxycoumarin, anisindione (e.g., MIRADON™), acenocoumarol (e.g., nicoumalone, SINTHROME™), indan-1,3-dione, phenprocoumon (e.g., MARCUMAR™), ethyl biscoumacetate (e.g., TROMEXAN™), and aspirin. In a specific embodiment, compositions of the invention are administered in combination with heparin and/or warfarin. In another specific embodiment, compositions of the invention are administered in combination with warfarin. In another specific embodiment, compositions of the invention are administered in combination with warfarin and aspirin. In another specific embodiment, compositions of the invention are administered in combination with heparin. In another specific embodiment, compositions of the invention are administered in combination with heparin and aspirin.

[0466] In another embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with thrombolytic drugs. Thrombolytic drugs that may be administered with the compositions of the invention include, but are not limited to, plasminogen, lys-plasminogen, alpha2-antiplasmin, streptokinase (e.g., KABIKINASE™), anistreplase (e.g., EMINASE™), tissue plasminogen activator (t-PA, alteplase, ACTIVASE™), urokinase (e.g., ABBOKINASE™), saruplase, (Prourokinase, single chain urokinase), and aminocaproic acid (e.g., AMICAR™). In a specific embodiment, compositions of the invention are administered in combination with tissue plasminogen activator and aspirin.

[0467] In another embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with antiplatelet drugs. Antiplatelet drugs that may be administered with the compositions of the invention include, but are not limited to, aspirin, dipyridamole (e.g., PERSANTINE™), and ticlopidine (e.g., TICLID™).

[0468] In specific embodiments, the use of anti-coagulants, thrombolytic and/or antiplatelet drugs in combination with albumin fusion proteins and/or polynucleotides of the invention is contemplated for the prevention, diagnosis, and/or treatment of thrombosis, arterial thrombosis, venous thrombosis, thromboembolism, pulmonary embolism, atherosclerosis, myocardial infarction, transient ischemic attack, unstable angina. In specific embodiments, the use of anticoagulants, thrombolytic drugs and/or antiplatelet drugs in combination with albumin fusion proteins and/or

polynucleotides of the invention is contemplated for the prevention of occlusion of saphenous grafts, for reducing the risk of periprocedural thrombosis as might accompany angioplasty procedures, for reducing the risk of stroke in patients with atrial fibrillation including nonrheumatic atrial fibrillation, for reducing the risk of embolism associated with mechanical heart valves and or mitral valves disease. Other uses for the therapeutics of the invention, alone or in combination with antiplatelet, anticoagulant, and/or thrombolytic drugs, include, but are not limited to, the prevention of occlusions in extracorporeal devices (e.g., intravascular canulas, vascular access shunts in hemodialysis patients, hemodialysis machines, and cardiopulmonary bypass machines).

[0469] In certain embodiments, albumin fusion proteins and/or polynucleotides of the invention are administered in combination with antiretroviral agents, nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and/or protease inhibitors (PIs). NRTIs that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). NNRTIs that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with albumin fusion proteins and/or polynucleotides of the invention to treat AIDS and/or to prevent or treat HIV infection.

[0470] Additional NRTIs include LODENOSINE™ (F-ddA; an acid-stable adenosine NRTI; Triangle/Abbott; COVIRACIL™ (emtricitabine/FTC; structurally related to lamivudine (3TC) but with 3- to 10-fold greater activity *in vitro*; Triangle/Abbott); dOTC (BCH-10652, also structurally related to lamivudine but retains activity against a substantial proportion of lamivudine-resistant isolates; Biochem Pharma); Adefovir (refused approval for anti-HIV therapy by FDA; Gilead Sciences); PREVEON® (Adefovir Dipivoxil, the active prodrug of adefovir; its active form is PMEA-pp); TENOFOVIR™ (bis-POC PMPA, a PMPA prodrug; Gilead); DAPD/DXG (active metabolite of DAPD; Triangle/Abbott); D-D4FC (related to 3TC, with activity against AZT/3TC-resistant virus); GW420867X (Glaxo Wellcome); ZIAGEN™ (abacavir/1592U89; Glaxo Wellcome Inc.); CS-87 (3'-azido-2',3'-dideoxyuridine; WO 99/66936); and S-acyl-2-thioethyl (SATE)-bearing prodrug forms of β-L-FD4C and β-L-FddC (WO 98/17281).

[0471] Additional NNRTIs include COACTINON™ (Emivirine/MKC-442, potent NNRTI of the HEPT class; Triangle/Abbott); CAPRAVIRINE™ (AG-1549/S-1153, a next generation NNRTI with activity against viruses containing the K103N mutation; Agouron); PNU-142721 (has 20- to 50-fold greater activity than its predecessor delavirdine and is active against K103N mutants; Pharmacia & Upjohn); DPC-961 and DPC-963 (second-generation derivatives of efavirenz, designed to be active against viruses with the K103N mutation; DuPont); GW-420867X (has 25-fold greater activity than HBY097 and is active against K103N mutants; Glaxo Wellcome); CALANOLIDE A (naturally occurring agent from the latex tree; active against viruses containing either or both the Y181C and K103N mutations); and Propolis (WO 99/49830).

[0472] Additional protease inhibitors include LOPINAVIR™ (ABT378/r; Abbott Laboratories); BMS-232632 (an azapeptide; Bristol-Myers Squibb); TIPRANAVIR™ (PNU-140690, a non-peptic dihydropyrone; Pharmacia & Upjohn); PD-178390 (a nonpeptidic dihydropyrone; Parke-Davis); BMS 232632 (an azapeptide; Bristol-Myers Squibb); L-756,423 (an indinavir analog; Merck); DMP-450 (a cyclic urea compound; Avid & DuPont); AG-1776 (a peptidomimetic with *in vitro* activity against protease inhibitor-resistant viruses; Agouron); VX-175/GW-433908 (phosphate prodrug of amprenavir; Vertex & Glaxo Wellcome); CGP61755 (Ciba); and AGENERASE™ (amprenavir; Glaxo Wellcome Inc.).

[0473] Additional antiretroviral agents include fusion inhibitors/gp41 binders. Fusion inhibitors/gp41 binders include T-20 (a peptide from residues 643-678 of the HIV gp41 transmembrane protein ectodomain which binds to gp41 in its resting state and prevents transformation to the fusogenic state; Trimeris) and T-1249 (a second-generation fusion inhibitor; Trimeris).

[0474] Additional antiretroviral agents include fusion inhibitors/chemokine receptor antagonists. Fusion inhibitors/chemokine receptor antagonists include CXCR4 antagonists such as AMD 3100 (a bicyclam), SDF-1 and its analogs, and ALX40-4C (a cationic peptide), T22 (an 18 amino acid peptide; Trimeris) and the T22 analogs T134 and T140; CCR5 antagonists such as RANTES (9-68), AOP-RANTES, NNY-RANTES, and TAK-779; and CCR5/CXCR4 antagonists such as NSC 651016 (a distamycin analog). Also included are CCR2B, CCR3, and CCR6 antagonists. Chemokine receptor agonists such as RANTES, SDF-1, MIP-1α, MIP-1β, etc., may also inhibit fusion.

[0475] Additional antiretroviral agents include integrase inhibitors. Integrase inhibitors include dicaffeoylquinic (DFQA) acids; L-chicoric acid (a dicaffeoyltartaric (DCTA) acid); quinalizarin (QLC) and related anthraquinones; ZINTEVIR™ (AR 177, an oligonucleotide that probably acts at cell surface rather than being a true integrase inhibitor; Arondex); and naphthols such as those disclosed in WO 98/50347.

[0476] Additional antiretroviral agents include hydroxyurea-like compounds such as BCX-34 (a purine nucleoside phosphorylase inhibitor; Biocryst); ribonucleotide reductase inhibitors such as DIDOX™ (Molecules for Health); inosine monophosphate dehydrogenase (IMPDH) inhibitors such as VX-497 (Vertex); and mycophenolic acids such as CellCept (mycophenolate mofetil; Roche).

[0477] Additional antiretroviral agents include inhibitors of viral integrase, inhibitors of viral genome nuclear translocation such as arylene bis(methylketone) compounds; inhibitors of HIV entry such as AOP-RANTES, NNY-RANTES, RANTES-IgG fusion protein, soluble complexes of RANTES and glycosaminoglycans (GAG), and AMD-3100; nucleocapsid zinc finger inhibitors such as dithiane compounds; targets of HIV Tat and Rev; and pharmacoenhancers such as ABT-378.

[0478] Other antiretroviral therapies and adjunct therapies include cytokines and lymphokines such as MIP-1α, MIP-1β, SDF-1α, IL-2, PROLEUKIN™ (aldesleukin/L2-7001; Chiron), IL-4, IL-10, IL-12, and IL-13; interferons such as IFN-alpha2a, IFN-alpha2b, or IFN-beta; *antagonists of TNFα, NFκB, GM-CSF, M-CSF, and IL-10; agents that modulate immune activation such as* cyclosporin and prednisone; vaccines such as Remune™ (HIV Immunogen), APL 400-003 (Apollon), recombinant gp120 and fragments, bivalent (B/E) recombinant envelope glycoprotein, rgp120CM235, MN rgp120, SF-2 rgp120, gp120/soluble CD4 complex, Delta JR-FL protein, branched synthetic peptide derived from discontinuous gp120 C3/C4 domain, fusion-competent immunogens, and Gag, Pol, Nef, and Tat vaccines; gene-based therapies such as genetic suppressor elements (GSEs; WO 98/54366), and intrakines (genetically modified CC chemokines targetted to the ER to block surface expression of newly synthesized CCR5 (Yang *et al., PNAS* 94:11567-72 (1997); Chen *et al., Nat. Med.* 3:1110-16 (1997)); antibodies such as the anti-CXCR4 antibody 12G5, the anti-CCR5 antibodies 2D7, 5C7, PA8, PA9, PA10, PA11, PA12, and PA14, the anti-CD4 antibodies Q4120 and RPA-T4, the anti-CCR3 antibody 7B11, the anti-gp120 antibodies 17b, 48d, 447-52D, 257-D, 268-D and 50.1, anti-Tat antibodies, anti-TNF-α antibodies, and monoclonal antibody 33A; aryl hydrocarbon (AH) receptor agonists and antagonists such as TCDD, 3,3',4,4',5-pentachlorobiphenyl, 3,3',4,4'-tetrachlorobiphenyl, and α-naphthoflavone (WO 98/30213); and antioxidants such as γ-L-glutamyl-L-cysteine ethyl ester (γ-GCE; WO 99/56764).

[0479] In a further embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, remantidine, maxamine, or thymalfasin. Specifically, interferon albumin fusion protein can be administered in combination with any of these agents. Moreover, interferon alpha albumin fusion protein can also be administered with any of these agents, and preferably, interferon alpha 2a or 2b albumin fusion protein can be administered with any of these agents. Furthermore, interferon beta albumin fusion protein can also be administered with any of these agents. Additionally, any of the IFN hybrids albumin fusion proteins can be administered in combination with any of these agents.

[0480] In a most preferred embodiment, interferon albumin fusion protein is administered in combination with ribavirin. In a further preferred embodiment, interferon alpha albumin fusion protein is administered in combination with ribavirin. In a further preferred embodiment, interferon alpha 2a albumin fusion protein is administered in combination with ribavirin. In a further preferred embodiment, interferon alpha 2b albumin fusion protein is administered in combination with ribavirin. In a further preferred embodiment, interferon beta albumin fusion protein is administered in combination with ribavirin. In a further preferred embodiment, hybrid interferon albumin fusion protein is administered in combination with ribavirin.

[0481] In other embodiments, albumin fusion proteins and/or polynucleotides of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat or prevent an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat or prevent an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat or prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat or prevent an opportunistic cytomegalovirus infection. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat or prevent an opportunistic fungal infection. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the

invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat or prevent an opportunistic bacterial infection.

[0482] In a further embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, amoxicillin, beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rapamycin, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamethoxazole, and vancomycin.

[0483] In other embodiments, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with immunostimulants. Immunostimulants that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, levamisole (e.g., ERGAMISOL™), isoprinosine (e.g. INOSIPLEX™), interferons (e.g. interferon alpha), and interleukins (e.g., IL-2).

[0484] In other embodiments, albumin fusion proteins and/or polynucleotides of the invention are administered in combination with immunosuppressive agents. Immunosuppressive agents that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells. Other immunosuppressive agents that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, prednisolone, methotrexate, thalidomide, methoxsalen, rapamycin, leflunomide, mizoribine (BREDININ™), brequinar, deoxyspergualin, and azaspirane (SKF 105685), ORTHOCLONE OKT® 3 (muromonab-CD3), SANDIMMUNE™, NEORAL™, SANDOYA™ (cyclosporine), PROGRAF® (FK506, tacrolimus), CELLCEPT® (mycophenolate mofetil, of which the active metabolite is mycophenolic acid), IMURAN™ (azathioprine), glucocorticosteroids, adrenocortical steroids such as DELTASONE™ (prednisone) and HYDELTRASOL™ (prednisolone), FOLEX™ and MEXATE™ (methotrxate), OXSORALEN-ULTRA™ (methoxsalen) and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

[0485] In an additional embodiment, albumin fusion proteins and/or polynucleotides of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, ATGAM™ (antithymocyte globulin), and GAMIMUNE™. In a specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

[0486] In another embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered alone or as part of a combination therapy, either in vivo to patients or in vitro to cells, for the treatment of cancer. In a specific embodiment, the albumin fusion proteins, particularly IL-2-albumin fusions, are administered repeatedly during passive immunotherapy for cancer, such as adoptive cell transfer therapy for metastatic melanoma as described in Dudley *et al.* (Science Express, 19 September 2002, at www.sciencexpress.org, hereby incorporated by reference in its entirety).

[0487] In certain embodiments, the albumin fusion proteins and/or polynucleotides of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, corticosteroids (e.g. betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, and triamcinolone), nonsteroidal anti-inflammatory drugs (e.g., diclofenac, diflunisal, etodolac, fenoprofen, floctafenine, flurbiprofen, ibuprofen, indomethacin, ketoprofen, meclofenamate, mefenamic acid, meloxicam, nabumetone, naproxen, oxaprozin, phenylbutazone, piroxicam, sulindac, tenoxicam, tiaprofenic acid, and tolmetin.), as well as antihistamines, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranylline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

[0488] In an additional embodiment, the compositions of the invention are administered alone or in combination with an anti-angiogenic agent. Anti-angiogenic agents that may be administered with the compositions of the invention include, but are not limited to, Angiostatin (Entremed, Rockville, MD), Troponin-1 (Boston Life Sciences, Boston, MA), anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel (Taxol), Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, VEGI, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

[0489] Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include

oxo transition metal complexes.

[0490] Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

[0491] Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

[0492] A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include, but are not limited to, platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, (1991)); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, (1992)); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, (1992)); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, (1990)); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, (1987)); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, (1987)); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4- chloroanthronilic acid disodium or "CCA"; (Takeuchi et al., Agents Actions 36:312-316, (1992)); and metalloproteinase inhibitors such as BB94.

[0493] Additional anti-angiogenic factors that may also be utilized within the context of the present invention include Thalidomide, (Celgene, Warren, NJ); Angiostatic steroid; AGM-1470 (H. Brem and J. Folkman *J Pediatr. Surg.* 28:445-51 (1993)); an integrin alpha v beta 3 antagonist (C. Storgard et al., *J Clin. Invest.* 103:47-54 (1999)); carboxynaminolmidazole; Carboxyamidotriazole (CAI) (National Cancer Institute, Bethesda, MD); Combretastatin A-4 (CA4P) (OXIGENE, Boston, MA); Squalamine (Magainin Pharmaceuticals, Plymouth Meeting, PA); TNP-470, (Tap Pharmaceuticals, Deerfield, IL); ZD-0101 AstraZeneca (London, UK); APRA (CT2584); Benefin, Byrostatin-1 (SC339555); CGP-41251 (PKC 412); CM101; Dexrazoxane (ICRF187); DMXAA; Endostatin; Flavopiridol; Genestein; GTE; ImmTher; Iressa (ZD1839); Octreotide (Somatostatin); Panretin; Penicillamine; Photopoint; PI-88; Primomastat (AG-3340) Purlytin; Suradista (FCE26644); Tamoxifen (Nolvadex); Tazarotene; Tetrathiomolybdate; Xeloda (Capecitabine); and 5-Fluorouracil.

[0494] Anti-angiogenic agents that may be administered in combination with the compounds of the invention may work through a variety of mechanisms including, but not limited to, inhibiting proteolysis of the extracellular matrix, blocking the function of endothelial cell-extracellular matrix adhesion molecules, by antagonizing the function of angiogenesis inducers such as growth factors, and inhibiting integrin receptors expressed on proliferating endothelial cells. Examples of anti-angiogenic inhibitors that interfere with extracellular matrix proteolysis and which may be administered in combination with the compositons of the invention include, but are not limited to, AG-3340 (Agouron, La Jolla, CA), BAY-12-9566 (Bayer, West Haven, CT), BMS-275291 (Bristol Myers Squibb, Princeton, NJ), CGS-27032A (Novartis, East Hanover, NJ), Marimastat (British Biotech, Oxford, UK), and Metastat (Aetema, St-Foy, Quebec). Examples of anti-angiogenic inhibitors that act by blocking the function of endothelial cell-extracellular matrix adhesion molecules and which may be administered in combination with the compositons of the invention include, but are not limited to, EMD-121974 (Merck KgaA Darmstadt, Germany) and Vitaxin (Ixsys, La Jolla, CA/Medimmune, Gaithersburg, MD). Examples of anti-angiogenic agents that act by directly antagonizing or inhibiting angiogenesis inducers and which may be administered in combination with the compositons of the invention include, but are not limited to, Angiozyme (Ribozyme, Boulder, CO), Anti-VEGF antibody (Genentech, S. San Francisco, CA), PTK-787/ZK-225846 (Novartis, Basel, Switzerland), SU-101 (Sugen, S. San Francisco, CA), SU-5416 (Sugen/ Pharmacia Upjohn, Bridgewater, NJ), and SU-6668 (Sugen). Other anti-angiogenic agents act to indirectly inhibit angiogenesis. Examples of indirect inhibitors of angiogenesis which may be administered in combination with the compositons of the invention include, but are not limited to, IM-862 (Cytran, Kirkland, WA), Interferon-alpha, IL-12 (Roche, Nutley, NJ), and Pentosan polysulfate (Georgetown University, Washington, DC).

[0495] In particular embodiments, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of an autoimmune disease, such as for example, an autoimmune disease described herein.

[0496] In a particular embodiment, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of arthritis. In a more particular embodiment, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of rheumatoid arthritis.

[0497] In another embodiment, the polynucleotides encoding a polypeptide of the present invention are administered in combination with an angiogenic protein, or polynucleotides encoding an angiogenic protein. Examples of angiogenic proteins that may be administered with the compositions of the invention include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2, VEGF-3, epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin-like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

[0498] In additional embodiments, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to alkylating agents such as nitrogen mustards (for example, Mechlorethamine, cyclophosphamide, Cyclophosphamide Ifosfamide, Melphalan (L-sarcolysin), and Chlorambucil), ethyleniminies and methylmelamines (for example, Hexamethylmelamine and Thiotepa), alkyl sulfonates (for example, Busulfan), nitrosoureas (for example, Carmustine (BCNU), Lomustine (CCNU), Semustine (methyl-CCNU), and Streptozocin (streptozotocin)), triazenes (for example, Dacarbazine (DTIC; dimethyltriazenoimidazolecarboxamide)), folic acid analogs (for example, Methotrexate (amethopterin)), pyrimidine analogs (for example, Fluorouacil (5-fluorouracil; 5-FU), Floxuridine (fluorodeoxyuridine; FudR), and Cytarabine (cytosine arabinoside)), purine analogs and related inhibitors (for example, Mercaptopurine (6-mercaptopurine; 6-MP), Thioguanine (6-thioguanine; TG), and Pentostatin (2'-deoxycoformycin)), vinca alkaloids (for example, Vinblastine (VLB, vinblastine sulfate)) and Vincristine (vincristine sulfate)), epipodophyllotoxins (for example, Etoposide and Teniposide), antibiotics (for example, Dactinomycin (actinomycin D), Daunorubicin (daunomycin; rubidomycin), Doxorubicin, Bleomycin, Plicamycin (mithramycin), and Mitomycin (mitomycin C), enzymes (for example, L-Asparaginase), biological response modifiers (for example, Interferon-alpha and interferon-alpha-2b), platinum coordination compounds (for example, Cisplatin (cis-DDP) and Carboplatin), anthracenedione (Mitoxantrone), substituted urea (for example, Hydroxyurea), methylhydrazine derivatives (for example, Procarbazine (N-methylhydrazine; MIH), adrenocorticosteroids (for example, Prednisone), progestins (for example, Hydroxyprogesterone caproate, Medroxyprogesterone, Medroxyprogesterone acetate, and Megestrol acetate), estrogens (for example, Diethylstilbestrol (DES), Diethylstilbestrol diphosphate, Estradiol, and Ethinyl estradiol), antiestrogens (for example, Tamoxifen), androgens (Testosterone proprionate, and Fluoxymesterone), antiandrogens (for example, Flutamide), gonadotropin-releasing hormone analogs (for example, Leuprolide), other hormones and hormone analogs (for example, methyltestosterone, estramustine, estramustine phosphate sodium, chlorotrianisene, and testolactone), and others (for example, dicarbazine, glutamic acid, and mitotane).

[0499] In one embodiment, the compositions of the invention are administered in combination with one or more of the following drugs: infliximab (also known as Remicade™ Centocor, Inc.), Trocade (Roche, RO-32-3555), Leflunomide (also known as Arava™ from Hoechst Marion Roussel), Kineret™ (an IL-1 Receptor antagonist also known as Anakinra from Amgen, Inc.)

[0500] In a specific embodiment, compositions of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or combination of one or more of the components of CHOP. In one embodiment, the compositions of the invention are administered in combination with anti-CD20 antibodies, human monoclonal anti-CD20 antibodies. In another embodiment, the compositions of the invention are administered in combination with anti-CD20 antibodies and CHOP, or anti-CD20 antibodies and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. In a specific embodiment, compositions of the invention are administered in combination with Rituximab. In a further embodiment, compositions of the invention are administered with Rituximab and CHOP, or Rituximab and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. In a specific embodiment, compositions of the invention are administered in combination with tositumomab. In a further embodiment, compositions of the invention are administered with tositumomab and CHOP, or tositumomab and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. The anti-CD20 antibodies may optionally be associated with radioisotopes, toxins or cytotoxic prodrugs.

[0501] In another specific embodiment, the compositions of the invention are administered in combination Zevalin™. In a further embodiment, compositions of the invention are administered with Zevalin™ and CHOP, or Zevalin™ and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. Zevalin™ may be associated with one or more radioisotopes. Particularly preferred isotopes are ^{90}Y and ^{111}In.

[0502] In an additional embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with cytokines. Cytokines that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment, albumin fusion proteins and/or polynucleotides of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

[0503] In one embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in

complex heterotrimer LT-alpha2-beta), ULBP1, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), OPG, and neutrokine-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TRANK, TR9 (International Publication No. WO 98/56892),TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

[0504] In an additional embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PlGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PlGF-2), as disclosed in Hauser et al., Growth Factors, 4:259-268 (1993); Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3); Vascular Endothelial Growth Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent Number DE19639601. The above mentioned references are herein incorporated by reference in their entireties.

[0505] In an additional embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

[0506] In an additional embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, granulocyte macrophage colony stimulating factor (GM-CSF) (sargramostim, LEUKINE™, PROKINE™), granulocyte colony stimulating factor (G-CSF) (filgrastim, NEUPOGEN™), macrophage colony stimulating factor (M-CSF, CSF-1) erythropoietin (epoetin alfa, EPOGEN™, PROCRIT™), stem cell factor (SCF, c-kit ligand, steel factor), megakaryocyte colony stimulating factor, PIXY321 (a GMCSF/IL-3 fusion protein), interleukins, especially any one or more of IL-1 through IL-12, interferon-gamma, or thrombopoietin.

[0507] In certain embodiments, albumin fusion proteins and/or polynucleotides of the present invention are administered in combination with adrenergic blockers, such as, for example, acebutolol, atenolol, betaxolol, bisoprolol, carteolol, labetalol, metoprolol, nadolol, esprenolol, penbutolol, pindolol, propranolol, sotalol, and timolol.

[0508] In another embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with an antiarrhythmic drug (e.g., adenosine, amiodarone, bretylium, digitalis, digoxin, digitoxin, diltiazem, disopyramide, esmolol, flecainide, lidocaine, mexiletine, moricizine, phenytoin, procainamide, N-acetyl procainamide, propafenone, propranolol, quinidine, sotalol, tocainide, and verapamil).

[0509] In another embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with diuretic agents, such as carbonic anhydrase-inhibiting agents (e.g., acetazolamide, dichlorphenamide, and methazolamide), osmotic diuretics (e.g., glycerin, isosorbide, mannitol, and urea), diuretics that inhibit Na^+-K^+-2Cl^- symport (e.g., furosemide, bumetanide, azosemide, piretanide, tripamide, ethacrynic acid, muzolimine, and torsemide), thiazide and thiazide-like diuretics (e.g., bendroflumethiazide, benzthiazide, chlorothiazide, hydrochlorothiazide, hydroflumethiazide, methyclothiazide, polythiazide, trichormethiazide, chlorthalidone, indapamide, metolazone, and quinethazone), potassium sparing diuretics (e.g., amiloride and triamterene), and mineralcorticoid receptor antagonists (e.g., spironolactone, canrenone, and potassium canrenoate).

[0510] In one embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with treatments for endocrine and/or hormone imbalance disorders. Treatments for endocrine and/or hormone imbalance disorders include, but are not limited to, ^{127}I, radioactive isotopes of iodine such as ^{131}I and ^{125}I; recombinant growth hormone, such as HUMATROPE™ (recombinant somatropin); growth hormone analogs such as PROTROPIN™ (somatrem); dopamine agonists such as PARLODEL™ (bromocriptine); somatostatin analogs such as SANDOSTATIN™ (octreotide); gonadotropin preparations such as PREGNYL™, A.P.L.™ and PROFASI™ (chorionic gonadotropin (CG)), PERGONAL™ (menotropins), and METRODIN™ (urofollitropin (uFSH)); synthetic human gonadotropin releasing hormone preparations such as FACTREL™ and LUTREPULSE™ (gonadorelin hydrochloride); synthetic gonadotropin agonists such as LUPRON™ (leuprolide acetate),

SUPPRELIN™ (histrelin acetate), SYNAREL™ (nafarelin acetate), and ZOLADEX™ (goserelin acetate); synthetic preparations of thyrotropin-releasing hormone such as RELEFACT TRH™ and THYPINONE™ (protirelin); recombinant human TSH such as THYROGEN™; synthetic preparations of the sodium salts of the natural isomers of thyroid hormones such as L-T_4™, SYNTHROID™ and LEVOTHROID™ (levothyroxine sodium), L-T_3™, CYTOMEL™ and TRIOSTAT™ (liothyroine sodium), and THYROLAR™ (liotrix); antithyroid compounds such as 6-n-propylthiouracil (propylthiouracil), 1-methyl-2-mercaptoimidazole and TAPAZOLE™ (methimazole), NEO-MERCAZOLE™ (carbimazole); beta-adrenergic receptor antagonists such as propranolol and esmolol; Ca^{2+} channel blockers; dexamethasone and iodinated radiological contrast agents such as TELEPAQUE™ (iopanoic acid) and ORAGRAFIN™ (sodium ipodate).

[0511] Additional treatments for endocrine and/or hormone imbalance disorders include, but are not limited to, estrogens or conjugated estrogens such as ESTRACE™ (estradiol), ESTINYL™ (ethinyl estradiol), PREMARIN™, ESTRATAB™, ORTHO-EST™, OGEN™ and estropipate (estrone), ESTROVIS™ (quinestrol), ESTRADERM™ (estradiol), DELESTROGEN™ and VALERGEN™ (estradiol valerate), DEPO-ESTRADIOL CYPIONATE™ and ESTROJECT LA™ (estradiol cypionate); antiestrogens such as NOLVADEX™ (tamoxifen), SEROPHENE™ and CLOMID™ (clomiphene); progestins such as DURALUTIN™ (hydroxyprogesterone caproate), MPA™ and DEPO-PROVERA™ (medroxyprogesterone acetate), PROVERA™ and CYCRIN™ (MPA), MEGACE™ (megestrol acetate), NORLUTIN™ (norethindrone), and NORLUTATE™ and AYGESTIN™ (norethindrone acetate); progesterone implants such as NORPLANT SYSTEM™ (subdermal implants of norgestrel); antiprogestins such as RU 486™ (mifepristone); hormonal contraceptives such as ENOVID™ (norethynodrel plus mestranol), PROGESTASERT™ (intrauterine device that releases progesterone), LOESTRIN™, BREVICON™, MODICON™, GENORA™, NELONA™, NORINYL™, OVACON-35™ and OVACON-50™ (ethinyl estradiol/norethindrone), LEVLEN™, NORDETTE™, TRI-LEVLEN™ and TRIPHASIL-21™ (ethinyl estradiol/levonorgestrel) LO/OVRAL™ and OVRAL™ (ethinyl estradiol/norgestrel), DEMULEN™ (ethinyl estradiol/ethynodiol diacetate), NORINYL™, ORTHO-NOVUM™, NORETHIN™, GENORA™, and NELOVA™ (norethindrone/mestranol), DESOGEN™ and ORTHO-CEPT™ (ethinyl estradiol/desogestrel), ORTHO-CYCLEN™ and ORTHO-TRICYCLEN™ (ethinyl estradiol/norgestimate), MICRONOR™ and NOR-QD™ (norethindrone), and OVRETTE™ (norgestrel).

[0512] Additional treatments for endocrine and/or hormone imbalance disorders include, but are not limited to, testosterone esters such as methenolone acetate and testosterone undecanoate; parenteral and oral androgens such as TESTOJECT-50™ (testosterone), TESTEX™ (testosterone propionate), DELATESTRYL™ (testosterone enanthate), DEPO-TESTOSTERONE™ (testosterone cypionate), DANOCRINE™ (danazol), HALOTESTIN™ (fluoxymesterone), ORETON METHYL™, TESTRED™ and VIRILON™ (methyltestosterone), and OXANDRIN™ (oxandrolone); testosterone transdermal systems such as TESTODERM™; androgen receptor antagonist and 5-alpha-reductase inhibitors such as ANDROCUR™ (cyproterone acetate), EULEXIN™ (flutamide), and PROSCAR™ (finasteride); adrenocorticotropic hormone preparations such as CORTROSYN™ (cosyntropin); adrenocortical steroids and their synthetic analogs such as ACLOVATE™ (alclometasone dipropionate), *CYCLOCORT™ (amcinonide), BECLOVENT™ and VANCERIL™ (beclomethasone dipropionate), CELESTONE™* (betamethasone), BENISONE™ and UTICORT™ (betamethasone benzoate), DIPROSONE™ (betamethasone dipropionate), CELESTONE PHOSPHATE™ (betamethasone sodium phosphate), CELESTONE SOLUSPAN™ (betamethasone sodium phosphate and acetate), BETA-VAL™ and VALISONE™ (betamethasone valerate), TEMOVATE™ (clobetasol propionate), CLODERM™ (clocortolone pivalate), CORTEF™ and HYDROCORTONE™ (cortisol (hydrocortisone)), HYDROCORTONE ACETATE™ (cortisol (hydrocortisone) acetate), LOCOID™ (cortisol (hydrocortisone) butyrate), HYDROCORTONE PHOSPHATE™ (cortisol (hydrocortisone) sodium phosphate), A-HYDROCORT™ and SOLU CORTEF™ (cortisol (hydrocortisone) sodium succinate), WESTCORT™ (cortisol (hydrocortisone) valerate), CORTISONE ACETATE™ (cortisone acetate), DESOWEN™ and TRIDESILON™ (desonide), TOPICORT™ (desoximetasone), DECADRON™ (dexamethasone), DECADRON LA™ (dexamethasone acetate), DECADRON PHOSPHATE™ and HEXADROL PHOSPHATE™ (dexamethasone sodium phosphate), FLORONE™ and MAXIFLOR™ (diflorasone diacetate), FLORINEF ACETATE™ (fludrocortisone acetate), AEROBID™ and NASALIDE™ (flunisolide), FLUONID™ and SYNALAR™ (fluocinolone acetonide), LIDEX™ (fluocinonide), FLUOR-OP™ and FML™ (fluorometholone), CORDRAN™ (flurandrenolide), HALOG™ (halcinonide), HMS LIZUIFILM™ (medrysone), MEDROL™ (methylprednisolone), DEPO-MEDROL™ and MEDROL ACETATE™ (methylprednisone acetate), A-METHAPRED™ and SOLUMEDROL™ (methylprednisolone sodium succinate), ELOCON™ (mometasone furoate), HALDRONE™ (paramethasone acetate), DELTA-CORTEF™ (prednisolone), ECONOPRED™ (prednisolone acetate), HYDELTRASOL™ (prednisolone sodium phosphate), HYDELTRA-T.B.A™ (prednisolone tebutate), DELTASONE™ (prednisone), ARISTOCORT™ and KENACORT™ (triamcinolone), KENALOG™ (triamcinolone acetonide), ARISTOCORT™ and KENACORT DIACETATE™ (triamcinolone diacetate), and ARISTOSPAN™ (triamcinolone hexacetonide); inhibitors of biosynthesis and action of adrenocortical steroids such as CYTADREN™ (aminoglutethimide), NIZORAL™ (ketoconazole), MODRASTANE™ (trilostane), and METOPIRONE™ (metyrapone); bovine, porcine or human insulin or mixtures thereof; insulin analogs; recombinant human insulin such as HUMULIN™ and NOVOLIN™; oral hypoglycemic agents such as ORAMIDE™ and ORINASE™ (tolbutamide), DIABINESE™ (chlorpropamide), TOLAMIDE™ and TOLINASE™ (tolazamide), DYMELOR™ (acetohexamide), glibenclamide, MICRONASE™, DIBETA™ and GLYNASE™

(glyburide), GLUCOTROL™ (glipizide), and DIAMICRON™ (gliclazide), GLUCOPHAGE™ (metformin), ciglitazone, pioglitazone, and alpha-glucosidase inhibitors; bovine or porcine glucagon; somatostatins such as SANDOSTATIN™ (octreotide); and diazoxides such as PROGLYCEM™ (diazoxide).

[0513] In one embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with treatments for uterine motility disorders. Treatments for uterine motility disorders include, but are not limited to, estrogen drugs such as conjugated estrogens (e.g., PREMARIN® and ESTRATAB®), estradiols (e.g., CLIMARA® and ALORA®), estropipate, and chlorotrianisene; progestin drugs (e.g., AMEN® (medroxyprogesterone), MICRONOR® (norethindrone acetate), PROMETRIUM® progesterone, and megestrol acetate); and estrogen/progesterone combination therapies such as, for example, conjugated estrogens/medroxyprogesterone (e.g., PREMPRO™ and PREMPHASE®) and norethindrone acetate/ethinyl estradiol (e.g., FEMHRT™).

[0514] In an additional embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with drugs effective in treating iron deficiency and hypochromic anemias, including but not limited to, ferrous sulfate (iron sulfate, FEOSOL™), ferrous fumarate (e.g., FEOSTAT™), ferrous gluconate (e.g., FERGON™), polysaccharide-iron complex (e.g., NIFEREX™), iron dextran injection (e.g., INFED™), cupric sulfate, pyridoxine, riboflavin, Vitamin B_{12}, cyanocobalamin injection (e.g., REDISOL™, RUBRAMIN PC™), hydroxocobalamin, folic acid (e.g., FOLVITE™), leucovorin (folinic acid, 5-CHOH4PteGlu, citrovorum factor) or WELLCOVORIN (Calcium salt of leucovorin), transferrin or ferritin.

[0515] In certain embodiments, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with agents used to treat psychiatric disorders. Psychiatric drugs that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, antipsychotic agents (e.g., chlorpromazine, chlorprothixene, clozapine, fluphenazine, haloperidol, loxapine, mesoridazine, molindone, olanzapine, perphenazine, pimozide, quetiapine, risperidone, thioridazine, thiothixene, trifluoperazine, and triflupromazine), antimanic agents (e.g., carbamazepine, divalproex sodium, lithium carbonate, and lithium citrate), antidepressants (e.g., amitriptyline, amoxapine, bupropion, citalopram, clomipramine, desipramine, doxepin, fluvoxamine, fluoxetine, imipramine, isocarboxazid, maprotiline, mirtazapine, nefazodone, nortriptyline, paroxetine, phenelzine, protriptyline, sertraline, tranylcypromine, trazodone, trimipramine, and venlafaxine), antianxiety agents (e.g., alprazolam, buspirone, chlordiazepoxide, clorazepate, diazepam, halazepam, lorazepam, oxazepam, and prazepam), and stimulants (e.g., d-amphetamine, methylphenidate, and pemoline).

[0516] In other embodiments, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with agents used to treat neurological disorders. Neurological agents that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, antiepileptic agents (e.g., carbamazepine, clonazepam, ethosuximide, phenobarbital, phenytoin, primidone, valproic acid, divalproex sodium, felbamate, gabapentin, lamotrigine, levetiracetam, oxcarbazepine, tiagabine, topiramate, zonisamide, diazepam, lorazepam, and clonazepam), antiparkinsonian agents (e.g., levodopa/carbidopa, selegiline, amantidine, bromocriptine, pergolide, repinirole, pramipexole, benztropine; biperiden; ethopropazine; procyclidine; trihexyphenidyl, tolcapone), and ALS therapeutics (e.g. riluzole).

[0517] In another embodiment, albumin fusion proteins and/or polynucleotides of the invention are administered in combination with vasodilating agents and/or calcium channel blocking agents. Vasodilating agents that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, Angiotensin Converting Enzyme (ACE) inhibitors (e.g., papaverine, isoxsuprine, benazepril, captopril, ciliazapril, enalapril, enalaprilat, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, spirapril, trandolapril, and zylidrin), and nitrates (e.g., isosorbide dinitrate, isosorbide mononitrate, and nitroglycerin). Examples of calcium channel blocking agents that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to amlodipine, bepridil, diltiazem, felodipine, flunarizine, isradipine, nicardipine, nifedipine, nimodipine, and verapamil.

[0518] In certain embodiments, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with treatments for gastrointestinal disorders. Treatments for gastrointestinal disorders that may be administered with the albumin fusion protein and/or polynucleotide of the invention include, but are not limited to, H_2 histamine receptor antagonists (e.g., TAGAMET™ (cimetidine), ZANTAC™ (ranitidine), PEPCID™ (famotidine), and AXID™ (nizatidine)); inhibitors of H^+, K^+ ATPase (e.g., PREVACID™ (lansoprazole) and PRILOSEC™ (omeprazole)); Bismuth compounds (e.g., PEPTO-BISMOL™ (bismuth subsalicylate) and DE-NOL™ (bismuth subcitrate)); various antacids; sucralfate; prostaglandin analogs (e.g. CYTOTEC™ (misoprostol)); muscarinic cholinergic antagonists; laxatives (e.g., surfactant laxatives, stimulant laxatives, saline and osmotic laxatives); antidiarrheal agents (e.g., LOMOTIL™ (diphenoxylate), MOTOFEN™ (diphenoxin), and IMODIUM™ (loperamide hydrochloride)), synthetic analogs of somatostatin such as SANDOSTATIN™ (octreotide), antiemetic agents (e.g., ZOFRAN™ (ondansetron), KYTRIL™ (granisetron hydrochloride), tropisetron, dolasetron, metoclopramide, chlorpromazine, perphenazine, prochlorperazine, proclethazine, thiethylperazine, trifluopromazine, domperidone, haloperidol, droperidol, trimethobenzamide, dexamethasone, methylprednisolone, dronabinol, and nabilone); D2 antagonists (e.g., metoclopramide, trimethobenzamide and chlorpromazine); bile salts; chenodeoxycholic acid; ursodeoxycholic acid; and pancreatic enzyme preparations such as pancreatin and pancrelipase.

[0519] In additional embodiments, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with

other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

[0520] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions comprising albumin fusion proteins of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Gene Therapy

[0521] Constructs encoding albumin fusion proteins of the invention can be used as a part of a gene therapy protocol to deliver therapeutically effective doses of the albumin fusion protein. A preferred approach for *in vivo* introduction of nucleic acid into a cell is by use of a viral vector containing nucleic acid, encoding an albumin fusion protein of the invention. Infection of cells with a viral vector has the advantage that a large proportion of the targeted cells can receive the nucleic acid. Additionally, molecules encoded within the viral vector, *e.g.*, by a cDNA contained in the viral vector, are expressed efficiently in cells which have taken up viral vector nucleic acid.

[0522] Retrovirus vectors and adeno-associated virus vectors can be used as a recombinant gene delivery system for the transfer of exogenous nucleic acid molecules encoding albumin fusion proteins *in vivo*. These vectors provide efficient delivery of nucleic acids into cells, and the transferred nucleic acids are stably integrated into the chromosomal DNA of the host. The development of specialized cell lines (termed "packaging cells") which produce only replication-defective retroviruses has increased the utility of retroviruses for gene therapy, and defective retroviruses are characterized for use in gene transfer for gene therapy purposes (for a review see Miller, A.D. (1990) *Blood* 76:27 1). A replication defective retrovirus can be packaged into virions which can be used to infect a target cell through the use of a helper virus by standard techniques. Protocols for producing recombinant retroviruses and for infecting cells *in vitro* or *in vivo* with such viruses can be found in Current Protocols in Molecular Biology, Ausubel, F.M. *et al.*, (eds.) Greene Publishing Associates, (1989), Sections 9.10-9.14 and other standard laboratory manuals.

[0523] Another viral gene delivery system useful in the present invention uses adenovirus-derived vectors. The genome of an adenovirus can be manipulated such that it encodes and expresses a gene product of interest but is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. See, for example, Berkner *et al.*, *BioTechniques* 6:616 (1988); Rosenfeld *et al.*, Science 252:431-434 (1991); and Rosenfeld *et al.*, *Cell* 68:143-155 (1992). Suitable adenoviral vectors derived from the adenovirus strain Ad type 5 d1324 or other strains of adenovirus (*e.g.*, Ad2, Ad3, Ad7 etc.) are known to those skilled in the art. Recombinant adenoviruses can be advantageous in certain circumstances in that they are not capable of infecting nondividing cells and can be used to infect a wide variety of cell types, including epithelial cells (Rosenfeld *et al.*, (1992) cited supra). Furthermore, the virus particle is relatively stable and amenable to purification and concentration, and as above, can be modified so as to affect the spectrum of infectivity. Additionally, introduced adenoviral DNA (and foreign DNA contained therein) is not integrated into the genome of a host cell but remains episomal, thereby avoiding potential problems that can occur as a result of insertional mutagenesis in situations where introduced DNA becomes integrated into the host genome (*e.g.*, retroviral DNA). Moreover, the carrying capacity of the adenoviral genome for foreign DNA is large (up to 8 kilobases) relative to other gene delivery vectors (Berkner *et al.*, cited supra; Haj-Ahmand *et al.*, J. Virol. 57:267 (1986)).

[0524] In another embodiment, non-viral gene delivery systems of the present invention rely on endocytic pathways for the uptake of the subject nucleotide molecule by the targeted cell. Exemplary gene delivery systems of this type include liposomal derived systems, poly-lysine conjugates, and artificial viral envelopes. In a representative embodiment, a nucleic acid molecule encoding an albumin fusion protein of the invention can be entrapped in liposomes bearing positive charges on their surface (*e.g.*, lipofectins) and (optionally) which are tagged with antibodies against cell surface antigens of the target tissue (Mizuno *et al.* (1992) *No Shinkei Geka* 20:547-5 5 1; PCT publication W091/06309; Japanese patent application 1047381; and European patent publication EP-A-43075).

[0525] Gene delivery systems for a gene encoding an albumin fusion protein of the invention can be introduced into a patient by any of a number of methods. For instance, a pharmaceutical preparation of the gene delivery system can be introduced systemically, *e.g.* by intravenous injection, and specific transduction of the protein in the target cells occurs predominantly from specificity of transfection provided by the gene delivery vehicle, cell-type or tissue-type expression due to the transcriptional regulatory sequences controlling expression of the receptor gene, or a combination thereof. In other embodiments, initial delivery of the recombinant gene is more limited with introduction into the animal being quite localized. For example, the gene delivery vehicle can be introduced by catheter (see U.S. Patent 5,328,470) or by Stereotactic injection (*e.g.* Chen *et al.* (1994) *PNAS* 91: 3 054-3 05 7). The pharmaceutical preparation of the gene therapy construct can consist essentially of the gene delivery system in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Where the albumin fusion protein can be produced intact from recombinant cells, *e.g.* retroviral vectors, the pharmaceutical preparation can comprise one or more cells which produce the albumin fusion protein.

Additional Gene Therapy Methods

[0526] Also encompassed by the invention are gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of an albumin fusion protein of the invention. This method requires a polynucleotide which codes for an albumin fusion protein of the present

invention operatively linked to a promoter and any other genetic elements necessary for the expression of the fusion protein by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

[0527] Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide encoding an albumin fusion protein of the present invention ex vivo, with the engineered cells then being provided to a patient to be treated with the fusion protein of the present invention. Such methods are well-known in the art. For example, see Belldegrun, A., et al., J. Natl. Cancer Inst. 85: 207-216 (1993); Ferrantini, M. et al., Cancer Research 53: 1107-1112 (1993); Ferrantini, M. et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura, H., et al., Cancer Research 50: 5102-5106 (1990); Santodonato, L., et al., Human Gene Therapy 7:1-10 (1996); Santodonato, L., et al., Gene Therapy 4:1246-1255 (1997); and Zhang, J.-F. et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

[0528] As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

[0529] In one embodiment, polynucleotides encoding the albumin fusion proteins of the present invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, polynucleotides encoding the albumin fusion proteins of the present invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

[0530] The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

[0531] Any strong promoter known to those skilled in the art can be used for driving the expression of the polynucleotide sequence. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the gene corresponding to the Therapeutic protein portion of the albumin fusion proteins of the invention.

[0532] Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

[0533] The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

[0534] For the naked nucleic acid sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

[0535] The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

[0536] The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

[0537] The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

[0538] In certain embodiments, the polynucleotide constructs are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA (1989) 86:6077-6081, which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem. (1990) 265:10189-10192, which is herein incorporated by reference), in functional form.

[0539] Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

[0540] Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication No. WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., P. Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

[0541] Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

[0542] For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15 degrees celcius. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

[0543] The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., Methods of Immunology (1983), 101:512-527, which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include Ca^{2+}-EDTA chelation (Papahadjopoulos et al., Biochim. Biophys. Acta (1975) 394:483; Wilson et al., Cell 17:77 (1979)); ether injection (Deamer, D. and Bangham, A., Biochim. Biophys. Acta 443:629 (1976); Ostro et al., Biochem. Biophys. Res. Commun. 76:836 (1977); Fraley et al., Proc. Natl. Acad. Sci. USA 76:3348 (1979)); detergent dialysis (Enoch, H. and Strittmatter, P., Proc. Natl. Acad. Sci. USA 76:145 (1979)); and reverse-phase evaporation (REV) (Fraley et al., J. Biol. Chem. 255:10431 (1980); Szoka, F. and Papahadjopoulos, D., Proc. Natl. Acad. Sci. USA 75:145 (1978); Schaefer-Ridder et al., Science 215:166 (1982)), which are herein incorporated by reference.

[0544] Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

[0545] U.S. Patent No. 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 provide methods for delivering DNA-cationic lipid complexes to mammals.

[0546] In certain embodiments, cells are engineered, *ex vivo* or *in vivo*, using a retroviral particle containing RNA which comprises a sequence encoding an albumin fusion protein of the present invention. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

[0547] The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and $CaPO_4$ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

[0548] The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding an albumin fusion protein of the present invention. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express a fusion protin of the present invention.

[0549] In certain other embodiments, cells are engineered, *ex vivo* or *in vivo*, with polynucleotide contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses fusion protein of the present invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz et al. Am. Rev. Respir. Dis.109:233-238 (1974)). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld, M. A. et al. (1991) Science 252:431-434; Rosenfeld et al., (1992) Cell 68:143-155). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green, M. et al. (1979) Proc. Natl. Acad. Sci. USA 76:6606).

[0550] Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel. 3:499-503 (1993); Rosenfeld et al., Cell 68:143-155 (1992); Engelhardt et al., Human Genet. Ther. 4:759-769 (1993); Yang et al., Nature Genet. 7:362-369 (1994); Wilson et al., Nature 365:691-692 (1993); and U.S. Patent No. 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively express Ela and Elb, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

[0551] Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

[0552] In certain other embodiments, the cells are engineered, ex vivo or *in vivo*, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, N., Curr. Topics in Microbiol. Immunol. 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

[0553] For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct. These viral particles are then used to transduce eukaryotic cells, either *ex vivo* or *in vivo*. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express a fusion protein of the invention.

[0554] Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding a polypeptide of the present invention) via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997;

International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), which are herein encorporated by reference. This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

[0555] Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5' end of the desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

[0556] The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

[0557] The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

[0558] The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

[0559] The polynucleotide encoding an albumin fusion protein of the present invention may contain a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

[0560] Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biolistic injectors, particle accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers (Kaneda et al., Science 243:375 (1989)).

[0561] A preferred method of local administration is by direct injection. Preferably, an albumin fusion protein of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries. Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.

[0562] Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

[0563] Therapeutic compositions useful in systemic administration, include fusion proteins of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site. In specific embodiments, suitable delivery vehicles for use with systemic administration comprise liposomes comprising albumin fusion proteins of the invention for targeting the vehicle to a particular site.

[0564] Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA 189:11277-11281, 1992, which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

[0565] Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs

administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian.

[0566] Albumin fusion proteins of the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly preferred.

Biological Activities

[0567] Albumin fusion proteins and/or polynucleotides encoding albumin fusion proteins of the present invention, can be used in assays to test for one or more biological activities. If an albumin fusion protein and/or polynucleotide exhibits an activity in a particular assay, it is likely that the Therapeutic protein corresponding to the fusion portein may be involved in the diseases associated with the biological activity. Thus, the fusion protein could be used to treat the associated disease.

[0568] In preferred embodiments, the present invention encompasses a method of treating a disease or disorder listed in the "Preferred Indication Y" column of Table 1 comprising administering to a patient in which such treatment, prevention or amelioration is desired an albumin fusion protein of the invention that comprises a Therapeutic protein portion corresponding to a Therapeutic protein disclosed in the "Therapeutic Protein X" column of Table 1 (in the same row as the disease or disorder to be treated is listed in the "Preferred Indication Y" column of Table 1) in an amount effective to treat, prevent or ameliorate the disease or disorder.

[0569] In a further preferred embodiment, the present invention encompasses a method of treating a disease or disorder listed for a particular Therapeutic protein in the "Preferred Indication:Y" column of Table 1 comprising administering to a patient in which such treatment, prevention or amelioration is desired an albumin fusion protein of the invention that comprises a Therapeutic protein portion corresponding to the Therapeutic protein for which the indications in the Examples are related in an amount effective to treat, prevent or ameliorate the disease or disorder.

[0570] Specifically contemplated by the present invention are albumin fusion proteins produced by a cell when encoded by the polynucleotides that encode SEQ ID NO:Y. When these polynucleotides are used to express the encoded protein from a cell, the cell's natural secretion and processing steps produces a protein that lacks the signal sequence explicitly listed in columns 4 and/or 11 of Table 2. The specific amino acid sequence of the listed signal sequence is shown in the specification or is well known in the art. Thus, most preferred embodiments of the present invention include the albumin fusion protein produced by a cell (which would lack the leader sequence shown in columns 4 and/or 11 of Table 2). Also most preferred are polypeptides comprising SEQ ID NO:Y without the specific leader sequence listed in columns 4 and/or 11 of Table 2. Compositions comprising these two preferred embodiments, including pharmaceutical compositions, are also preferred. These albumin fusion proteins are specifically contemplated to treat, prevent, or ameliorate a disease or disorder listed for a particular Therapeutic protein in the "Preferred Indication:Y" column of Table 1.

[0571] In preferred embodiments, fusion proteins of the present invention may be used in the diagnosis, prognosis, prevention and/or treatment of diseases and/or disorders relating to diseases and disorders of the endocrine system (see, for example, "Endocrine Disorders" section below), the nervous system (see, for example, "Neurological Disorders" section below), the immune system (see, for example, "Immune Activity" section below), respiratory system (see, for example, "Respiratory Disorders" section below), cardiovascular system (see, for example, "Cardiovascular Disorders" section below), reproductive system (see, for example, "Reproductive System Disorders" section below) digestive system (see, for example, "Gastrointestinal Disorders" section below), diseases and/or disorders relating to cell proliferation (see, for example, "Hyperproliferative Disorders" section below), and/or diseases or disorders relating to the blood (see, for example, "Blood-Related Disorders" section below).

[0572] In certain embodiments, an albumin fusion protein of the present invention may be used to diagnose and/or prognose diseases and/or disorders associated with the tissue(s) in which the gene corresponding to the Therapeutic protein portion of the fusion protein of the invention is expressed.

[0573] Thus, fusion proteins of the invention and polynucleotides encoding albumin fusion proteins of the invention are useful in the diagnosis, detection and/or treatment of diseases and/or disorders associated with activities that include, but are not limited to, prohormone activation, neurotransmitter activity, cellular signaling, cellular proliferation, cellular differentiation, and cell migration.

[0574] More generally, fusion proteins of the invention and polynucleotides encoding albumin fusion proteins of the invention may be useful for the diagnosis, prognosis, prevention and/or treatment of diseases and/or disorders associated with the following systems.

Immune Activity

[0575] Albumin fusion proteins of the invention and polynucleotides encoding albumin fusion proteins of the invention may be useful in treating, preventing, diagnosing and/or prognosing diseases, disorders, and/or conditions of the immune system, by, for example, activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune diseases, disorders, and/or conditions may be genetic, somatic, such as cancer and some autoimmune diseases, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention can be used as a marker or detector of a particular immune system disease or disorder.

[0576] In another embodiment, a fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention, may

be used to treat diseases and disorders of the immune system and/or to inhibit or enhance an immune response generated by cells associated with the tissue(s) in which the polypeptide of the invention is expressed.

[0577] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in treating, preventing, diagnosing, and/or prognosing immunodeficiencies, including both congenital and acquired immunodeficiencies. Examples of B cell immunodeficiencies in which immunoglobulin levels B cell function and/or B cell numbers are decreased include: X-linked agammaglobulinemia (Bruton's disease), X-linked infantile agammaglobulinemia, X-linked immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, X-linked lymphoproliferative syndrome (XLP), agammaglobulinemia including congenital and acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, unspecified hypogammaglobulinemia, recessive agammaglobulinemia (Swiss type), Selective IgM deficiency, selective IgA deficiency, selective IgG subclass deficiencies, IgG subclass deficiency (with or without IgA deficiency), Ig deficiency with increased IgM, IgG and IgA deficiency with increased IgM, antibody deficiency with normal or elevated Igs, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), common variable immunodeficiency (CVID), common variable immunodeficiency (CVI) (acquired), and transient hypogammaglobulinemia of infancy.

[0578] In specific embodiments, ataxia-telangiectasia or conditions associated with ataxia-telangiectasia are treated, prevented, diagnosed, and/or prognosing using the, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention.

[0579] Examples of congenital immunodeficiencies in which T cell and/or B cell function and/or number is decreased include, but are not limited to: DiGeorge anomaly, severe combined immunodeficiencies (SCID) (including, but not limited to, X-linked SCID, autosomal recessive SCID, adenosine deaminase deficiency, purine nucleoside phosphorylase (PNP) deficiency, Class II MHC deficiency (Bare lymphocyte syndrome), Wiskott-Aldrich syndrome, and ataxia telangiectasia), thymic hypoplasia, third and fourth pharyngeal pouch syndrome, 22q11.2 deletion, chronic mucocutaneous candidiasis, natural killer cell deficiency (NK), idiopathic CD4+ T-lymphocytopenia, immunodeficiency with predominant T cell defect (unspecified), and unspecified immunodeficiency of cell mediated immunity.

[0580] In specific embodiments, DiGeorge anomaly or conditions associated with DiGeorge anomaly are treated, prevented, diagnosed, and/or prognosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention.

[0581] Other immunodeficiencies that may be treated, prevented, diagnosed, and/or prognosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, include, but are not limited to, chronic granulomatous disease, Chédiak-Higashi syndrome, myeloperoxidase deficiency, leukocyte glucose-6-phosphate dehydrogenase deficiency, X-linked lymphoproliferative syndrome (XLP), leukocyte adhesion deficiency, complement component deficiencies (including C1, C2, C3, C4, C5, C6, C7, C8 and/or C9 deficiencies), reticular dysgenesis, thymic alymphoplasia-aplasia, immunodeficiency with thymoma, severe congenital leukopenia, dysplasia with immunodeficiency, neonatal neutropenia, short limbed dwarfism, and Nezelof syndrome-combined immunodeficiency with Igs.

[0582] In a preferred embodiment, the immunodeficiencies and/or conditions associated with the immunodeficiencies recited above are treated, prevented, diagnosed and/or prognosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention.

[0583] In a preferred embodiment fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention could be used as an agent to boost immunoresponsiveness among immunodeficient individuals. In specific embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention could be used as an agent to boost immunoresponsiveness among B cell and/or T cell immunodeficient individuals.

[0584] The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in treating, preventing, diagnosing and/or prognosing autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

[0585] Autoimmune diseases or disorders that may be treated, prevented, diagnosed and/or prognosed by fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, one or more of the following: systemic lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, multiple sclerosis, autoimmune thyroiditis, Hashimoto's thyroiditis, autoimmune hemolytic anemia, hemolytic anemia, thrombocytopenia, autoimmune thrombocytopenia purpura, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, purpura (e.g., Henloch-Scoenlein purpura), autoimmunocytopenia, Goodpasture's syndrome, Pemphigus vulgaris, myasthenia gravis, Grave's disease (hyperthyroidism), and insulin-resistant diabetes mellitus.

[0586] Additional disorders that are likely to have an autoimmune component that may be treated, prevented, and/or diagnosed with the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, type II collagen-induced arthritis, antiphospholipid syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart

disease, neuritis, uveitis opthalmia, polyendocrinopathies, Reiter's Disease, Stiff-Man Syndrome, autoimmune pulmonary inflammation, autism, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disorders.

[0587] Additional disorders that are likely to have an autoimmune component that may be treated, prevented, diagnosed and/or prognosed with the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, scleroderma with anti-collagen antibodies (often characterized, e.g., by nucleolar and other nuclear antibodies), mixed connective tissue disease (often characterized, e.g., by antibodies to extractable nuclear antigens (e.g., ribonucleoprotein)), polymyositis (often characterized, e.g., by nonhistone ANA), pernicious anaemia (often characterized, e.g., by antiparietal cell, microsomes, and intrinsic factor antibodies), idiopathic Addison's disease (often characterized, e.g., by humoral and cell-mediated adrenal cytotoxicity, infertility (often characterized, e.g., by antispermatozoal antibodies), glomerulonephritis (often characterized, e.g., by glomerular basement membrane antibodies or immune complexes), bullous pemphigoid (often characterized, e.g., by IgG and complement in basement membrane), Sjogren's syndrome (often characterized, e.g., by multiple tissue antibodies, and/or a specific nonhistone ANA (SS-B)), diabetes mellitus (often characterized, e.g., by cell-mediated and humoral islet cell antibodies), and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis) (often characterized, e.g., by beta-adrenergic receptor antibodies).

[0588] Additional disorders that may have an autoimmune component that may be treated, prevented, diagnosed and/or prognosed with the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, chronic active hepatitis (often characterized, e.g., by smooth muscle antibodies), primary biliary cirrhosis (often characterized, e.g., by mitochondria antibodies), other endocrine gland failure (often characterized, e.g., by specific tissue antibodies in some cases), vitiligo (often characterized, e.g., by melanocyte antibodies), vasculitis (often characterized, e.g., by Ig and complement in vessel walls and/or low serum complement), post-MI (often characterized, e.g., by myocardial antibodies), cardiotomy syndrome (often characterized, e.g., by myocardial antibodies), urticaria (often characterized, e.g., by IgG and IgM antibodies to IgE), atopic dermatitis (often characterized, e.g., by IgG and IgM antibodies to IgE), asthma (often characterized, e.g., by IgG and IgM antibodies to IgE), and many other inflammatory, granulomatous, degenerative, and atrophic disorders.

[0589] In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, diagnosed and/or prognosed using for example, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention. In a specific preferred embodiment, rheumatoid arthritis is treated, prevented, and/or diagnosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention.

[0590] In another specific preferred embodiment, systemic lupus erythematosus is treated, prevented, and/or diagnosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention. In another specific preferred embodiment, idiopathic thrombocytopenia purpura is treated, prevented, and/or diagnosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention.

[0591] In another specific preferred embodiment IgA nephropathy is treated, prevented, and/or diagnosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention.

[0592] In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, diagnosed and/or prognosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention.

[0593] In preferred embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a immunosuppressive agent(s).

[0594] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in treating, preventing, prognosing, and/or diagnosing diseases, disorders, and/or conditions of hematopoietic cells. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with a decrease in certain (or many) types hematopoietic cells, including but not limited to, leukopenia, neutropenia, anemia, and thrombocytopenia. Alternatively, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with an increase in certain (or many) types of hematopoietic cells, including but not limited to, histiocytosis.

[0595] Allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated, prevented, diagnosed and/or prognosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention. Moreover, these molecules can be used to treat, prevent, prognose, and/or diagnose anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

[0596] Additionally, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may be used to treat, prevent, diagnose and/or prognose IgE-mediated allergic reactions. Such allergic reactions include, but are not limited to, asthma, rhinitis, and eczema. In specific embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be

used to modulate IgE concentrations in vitro or in vivo.

[0597] Moreover, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention have uses in the diagnosis, prognosis, prevention, and/or treatment of inflammatory conditions. For example, since fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may inhibit the activation, proliferation and/or differentiation of cells involved in an inflammatory response, these molecules can be used to prevent and/or treat chronic and acute inflammatory conditions. Such inflammatory conditions include, but are not limited to, for example, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome), ischemia-reperfusion injury, endotoxin lethality, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, over production of cytokines (e.g., TNF or IL-1.), respiratory disorders (e.g., asthma and allergy); gastrointestinal disorders (e.g., inflammatory bowel disease); cancers (e.g., gastric, ovarian, lung, bladder, liver, and breast); CNS disorders (e.g., multiple sclerosis; ischemic brain injury and/or stroke, traumatic brain injury, neurodegenerative disorders (e.g., Parkinson's disease and Alzheimer's disease); AIDS-related dementia; and prion disease); cardiovascular disorders (e.g., atherosclerosis, myocarditis, cardiovascular disease, and cardiopulmonary bypass complications); as well as many additional diseases, conditions, and disorders that are characterized by inflammation (e.g., hepatitis, rheumatoid arthritis, gout, trauma, pancreatitis, sarcoidosis, dermatitis, renal ischemia-reperfusion injury, Grave's disease, systemic lupus erythematosus, diabetes mellitus, and allogenic transplant rejection).

[0598] Because inflammation is a fundamental defense mechanism, inflammatory disorders can effect virtually any tissue of the body. Accordingly, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, have uses in the treatment of tissue-specific inflammatory disorders, including, but not limited to, adrenalitis, alveolitis, angiocholecystitis, appendicitis, balanitis, blepharitis, bronchitis, bursitis, carditis, cellulitis, cervicitis, cholecystitis, chorditis, cochlitis, colitis, conjunctivitis, cystitis, dermatitis, diverticulitis, encephalitis, endocarditis, esophagitis, eustachitis, fibrositis, folliculitis, gastritis, gastroenteritis, gingivitis, glossitis, hepatosplenitis, keratitis, labyrinthitis, laryngitis, lymphangitis, mastitis, media otitis, meningitis, metritis, mucitis, myocarditis, myosititis, myringitis, nephritis, neuritis, orchitis, osteochondritis, otitis, pericarditis, perichondritis, peritonitis, pharyngitis, phlebitis, policencyalitis, prostatitis, pulpitis, retinitis, rhinitis, salpingitis, scleritis, sclerochoroiditis, scrotitis, sinusitis, spondylitis, steatitis, stomatitis, synovitis, syringitis, tendonitis, tonsillitis, urethritis, and vaginitis.

[0599] In specific embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, are useful to diagnose, prognose, prevent, and/or treat organ transplant rejections and graft-versus-host disease. Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. Polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, that inhibit an immune response, particularly the activation, proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD. In specific embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, that inhibit an immune response, particularly the activation, proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing experimental allergic and hyperacute xenograft rejection.

[0600] In other embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, are useful to diagnose, prognose, prevent, and/or treat immune complex diseases, including, but not limited to, serum sickness, post streptococcal glomerulonephritis, polyarteritis nodosa, and immune complex-induced vasculitis.

[0601] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention can be used to treat, detect, and/or prevent infectious agents. For example, by increasing the immune response, particularly increasing the proliferation activation and/or differentiation of B and/or T cells, infectious diseases may be treated, detected, and/or prevented. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may also directly inhibit the infectious agent (refer to section of application listing infectious agents, etc), without necessarily eliciting an immune response.

[0602] In another embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a vaccine adjuvant that enhances immune responsiveness to an antigen. In a specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an adjuvant to enhance tumor-specific immune responses.

[0603] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an adjuvant to enhance anti-viral immune responses. Anti-viral immune responses that may be enhanced using the compositions of the invention as an adjuvant, include virus and virus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: AIDS, meningitis, Dengue, EBV, and hepatitis (e.g., hepatitis B). In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: HIV/AIDS, respiratory syncytial virus, Dengue, rotavirus, Japanese B encephalitis, Influenza A and B, parainfluenza, measles,

cytomegalovirus, rabies, Junin, Chikungunya, Rift Valley Fever, herpes simplex, and yellow fever.

[0604] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an adjuvant to enhance anti-bacterial or anti-fungal immune responses. Anti-bacterial or anti-fungal immune responses that may be enhanced using the compositions of the invention as an adjuvant, include bacteria or fungus and bacteria or fungus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: tetanus, Diphtheria, botulism, and *meningitis* type B.

[0605] In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: *Vibrio cholerae*, *Mycobacterium leprae*, *Salmonella typhi*, *Salmonella paratyphi*, *Meiseria meningitidis*, *Streptococcus pneumoniae*, Group B streptococcus, *Shigella spp.*, Enterotoxigenic *Escherichia coli*, Enterohemorrhagic *E. coli*, and *Borrelia burgdorferi*.

[0606] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an adjuvant to enhance anti-parasitic immune responses. Anti-parasitic immune responses that may be enhanced using the compositions of the invention as an adjuvant, include parasite and parasite associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a parasite. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to Plasmodium (malaria) or Leishmania.

[0607] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may also be employed to treat infectious diseases including silicosis, sarcoidosis, and idiopathic pulmonary fibrosis; for example, by preventing the recruitment and activation of mononuclear phagocytes.

[0608] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an antigen for the generation of antibodies to inhibit or enhance immune mediated responses against polypeptides of the invention.

[0609] In one embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are administered to an animal (e.g., mouse, rat, rabbit, hamster, guinea pig, pigs, micro-pig, chicken, camel, goat, horse, cow, sheep, dog, cat, non-human primate, and human, most preferably human) to boost the immune system to produce increased quantities of one or more antibodies (e.g., IgG, IgA, IgM, and IgE), to induce higher affinity antibody production and immunoglobulin class switching (e.g., IgG, IgA, IgM, and IgE), and/or to increase an immune response.

[0610] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a stimulator of B cell responsiveness to pathogens.

[0611] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an activator of T cells.

[0612] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an agent that elevates the immune status of an individual prior to their receipt of immunosuppressive therapies.

[0613] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an agent to induce higher affinity antibodies.

[0614] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an agent to increase serum immunoglobulin concentrations.

[0615] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an agent to accelerate recovery of immunocompromised individuals.

[0616] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an agent to boost immunoresponsiveness among aged populations and/or neonates.

[0617] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an immune system enhancer prior to, during, or after bone marrow transplant and/or other transplants (e.g., allogeneic or xenogeneic organ transplantation). With respect to transplantation, compositions of the invention may be administered prior to, concomitant with, and/or after transplantation. In a specific embodiment, compositions of the invention are administered after transplantation, prior to the beginning of recovery of T-cell populations. In another specific embodiment, compositions of the invention are first administered after transplantation after the beginning of recovery of T cell populations, but prior to full recovery of B cell populations.

[0618] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an agent to boost immunoresponsiveness among individuals having an acquired loss of B cell function. Conditions resulting in an acquired loss of B cell function that may be ameliorated or treated by administering the albumin fusion proteins of the invention

and/or polynucleotides encoding albumin fusion proteins of the invention, include, but are not limited to, HIV infection, AIDS, bone marrow transplant, and B cell chronic lymphocytic leukemia (CLL).

[0619] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an agent to boost immunoresponsiveness among individuals having a temporary immune deficiency. Conditions resulting in a temporary immune deficiency that may be ameliorated or treated by administering the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, include, but are not limited to, recovery from viral infections (e.g., influenza), conditions associated with malnutrition, recovery from infectious mononucleosis, or conditions associated with stress, recovery from measles, recovery from blood transfusion, and recovery from surgery.

[0620] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a regulator of antigen presentation by monocytes, dendritic cells, and/or B-cells. In one embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention enhance antigen presentation or antagonize antigen presentation in vitro or in vivo. Moreover, in related embodiments, this enhancement or antagonism of antigen presentation may be useful as an anti-tumor treatment or to modulate the immune system.

[0621] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an agent to direct an individual's immune system towards development of a humoral response (i.e. TH2) as opposed to a TH1 cellular response.

[0622] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a means to induce tumor proliferation and thus make it more susceptible to anti-neoplastic agents. For example, multiple myeloma is a slowly dividing disease and is thus refractory to virtually all anti-neoplastic regimens. If these cells were forced to proliferate more rapidly their susceptibility profile would likely change.

[0623] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a stimulator of B cell production in pathologies such as AIDS, chronic lymphocyte disorder and/or Common Variable Immunodificiency.

[0624] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a therapy for generation and/or regeneration of lymphoid tissues following surgery, trauma or genetic defect. In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used in the pretreatment of bone marrow samples prior to transplant.

[0625] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a gene-based therapy for genetically inherited disorders resulting in immuno-incompetence/immunodeficiency such as observed among SCID patients.

[0626] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a means of activating monocytes/macrophages to defend against parasitic diseases that effect monocytes such as Leishmania.

[0627] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a means of regulating secreted cytokines that are elicited by polypeptides of the invention.

[0628] In another embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used in one or more of the applications decribed herein, as they may apply to veterinary medicine.

[0629] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a means of blocking various aspects of immune responses to foreign agents or self. Examples of diseases or conditions in which blocking of certain aspects of immune responses may be desired include autoimmune disorders such as lupus, and arthritis, as well as immunoresponsiveness to skin allergies, inflammation, bowel disease, injury and diseases/disorders associated with pathogens.

[0630] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a therapy for preventing the B cell proliferation and Ig secretion associated with autoimmune diseases such as idiopathic thrombocytopenic purpura, systemic lupus erythematosus and multiple sclerosis.

[0631] In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention invention are used as a inhibitor of B and/or T cell migration in endothelial cells. This activity disrupts tissue architecture or cognate responses and is useful, for example in disrupting immune responses, and blocking sepsis.

[0632] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a therapy for chronic hypergammaglobulinemia evident in such diseases as monoclonal gammopathy of undetermined significance (MGUS), Waldenstrom's disease, related idiopathic monoclonal gammopathies, and plasmacytomas.

[0633] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the

invention may be employed for instance to inhibit polypeptide chemotaxis and activation of macrophages and their precursors, and of neutrophils, basophils, B lymphocytes and some T-cell subsets, e.g., activated and CD8 cytotoxic T cells and natural killer cells, in certain autoimmune and chronic inflammatory and infective diseases. Examples of autoimmune diseases are described herein and include multiple sclerosis, and insulin-dependent diabetes.

[0634] The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may also be employed to treat idiopathic hyper-eosinophilic syndrome by, for example, preventing eosinophil production and migration.

[0635] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to enhance or inhibit complement mediated cell lysis.

[0636] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to enhance or inhibit antibody dependent cellular cytotoxicity.

[0637] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may also be employed for treating atherosclerosis, for example, by preventing monocyte infiltration in the artery wall.

[0638] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be employed to treat adult respiratory distress syndrome (ARDS).

[0639] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful for stimulating wound and tissue repair, stimulating angiogenesis, and/or stimulating the repair of vascular or lymphatic diseases or disorders. Additionally, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to stimulate the regeneration of mucosal surfaces.

[0640] In a specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to diagnose, prognose, treat, and/or prevent a disorder characterized by primary or acquired immunodeficiency, deficient serum immunoglobulin production, recurrent infections, and/or immune system dysfunction. Moreover, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to treat or prevent infections of the joints, bones, skin, and/or parotid glands, blood-borne infections (e.g., sepsis, meningitis, septic arthritis, and/or osteomyelitis), autoimmune diseases (e.g., those disclosed herein), inflammatory disorders, and malignancies, and/or any disease or disorder or condition associated with these infections, diseases, disorders and/or malignancies) including, but not limited to, CVID, other primary immune deficiencies, HIV disease, CLL, recurrent bronchitis, sinusitis, otitis media, conjunctivitis, pneumonia, hepatitis, meningitis, herpes zoster (e.g., severe herpes zoster), and/or pneumocystis carnii. Other diseases and disorders that may be prevented, diagnosed, prognosed, and/or treated with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, HIV infection, HTLV-BLV infection, lymphopenia, phagocyte bactericidal dysfunction anemia, thrombocytopenia, and hemoglobinuria.

[0641] In another embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat, and/or diagnose an individual having common variable immunodeficiency disease ("CVID"; also known as "acquired agammaglobulinemia" and "acquired hypogammaglobulinemia") or a subset of this disease.

[0642] In a specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to diagnose, prognose, prevent, and/or treat cancers or neoplasms including immune cell or immune tissue-related cancers or neoplasms. Examples of cancers or neoplasms that may be prevented, diagnosed, or treated by fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, acute myelogenous leukemia, chronic myelogenous leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, acute lymphocytic anemia (ALL) Chronic lymphocyte leukemia, plasmacytomas, multiple myeloma, Burkitt's lymphoma, EBV-transformed diseases, and/or diseases and disorders described in the section entitled "Hyperproliferative Disorders" elsewhere herein.

[0643] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a therapy for decreasing cellular proliferation of Large B-cell Lymphomas.

[0644] In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a means of decreasing the involvement of B cells and Ig associated with Chronic Myelogenous Leukemia.

[0645] In specific embodiments, the compositions of the invention are used as an agent to boost immunoresponsiveness among B cell immunodeficient individuals, such as, for example, an individual who has undergone a partial or complete splenectomy.

Blood-Related Disorders

[0646] The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to modulate hemostatic (the stopping of bleeding) or thrombolytic (clot dissolving) activity. For example, by increasing hemostatic or thrombolytic activity, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention could be used to treat or prevent blood coagulation diseases, disorders, and/or conditions (e.g., afibrinogenemia, factor deficiencies, hemophilia), blood platelet diseases, disorders, and/or conditions (e.g., thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, fusion proteins of the

invention and/or polynucleotides encoding albumin fusion proteins of the invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment or prevention of heart attacks (infarction), strokes, or scarring.

[0647] In specific embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to prevent, diagnose, prognose, and/or treat thrombosis, arterial thrombosis, venous thrombosis, thromboembolism, pulmonary embolism, atherosclerosis, myocardial infarction, transient ischemic attack, unstable angina. In specific embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used for the prevention of occlusion of saphenous grafts, for reducing the risk of periprocedural thrombosis as might accompany angioplasty procedures, for reducing the risk of stroke in patients with atrial fibrillation including nonrheumatic atrial fibrillation, for reducing the risk of embolism associated with mechanical heart valves and or mitral valves disease. Other uses for the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, include, but are not limited to, the prevention of occlusions in extracorporeal devices (e.g., intravascular canulas, vascular access shunts in hemodialysis patients, hemodialysis machines, and cardiopulmonary bypass machines).

[0648] In another embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may be used to prevent, diagnose, prognose, and/or treat diseases and disorders of the blood and/or blood forming organs associated with the tissue(s) in which the polypeptide of the invention is expressed.

[0649] The fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to modulate hematopoietic activity (the formation of blood cells). For example, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to increase the quantity of all or subsets of blood cells, such as, for example, erythrocytes, lymphocytes (B or T cells), myeloid cells (e.g., basophils, eosinophils, neutrophils, mast cells, macrophages) and platelets. The ability to decrease the quantity of blood cells or subsets of blood cells may be useful in the prevention, detection, diagnosis and/or treatment of anemias and leukopenias described below. Alternatively, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to decrease the quantity of all or subsets of blood cells, such as, for example, erythrocytes, lymphocytes (B or T cells), myeloid cells (e.g., basophils, eosinophils, neutrophils, mast cells, macrophages) and platelets.. The ability to decrease the quantity of blood cells or subsets of blood cells may be useful in the prevention, detection, diagnosis and/or treatment of leukocytoses, such as, for example eosinophilia.

[0650] The fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to prevent, treat, or diagnose blood dyscrasia.

[0651] Anemias are conditions in which the number of red blood cells or amount of hemoglobin (the protein that carries oxygen) in them is below normal. Anemia may be caused by excessive bleeding, decreased red blood cell production, or increased red blood cell destruction (hemolysis). The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in treating, preventing, and/or diagnosing anemias. Anemias that may be treated prevented or diagnosed by the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include iron deficiency anemia, hypochromic anemia, microcytic anemia, chlorosis, hereditary sideroblastic anemia, idiopathic acquired sideroblastic anemia, red cell aplasia, megaloblastic anemia (e.g., pernicious anemia, (vitamin B12 deficiency) and folic acid deficiency anemia), aplastic anemia, hemolytic anemias (e.g., autoimmune hetolytic anemia, microangiopathic hemolytic anemia, and paroxysmal nocturnal hemoglobinuria). The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in treating, preventing, and/or diagnosing anemias associated with diseases including but not limited to, anemias associated with systemic lupus erythematosus, cancers, lymphomas, chronic renal disease, and enlarged spleens. The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in treating, preventing, and/or diagnosing anemias arising from drug treatments such as anemias associated with methyldopa, dapsone, and/or sulfadrugs. Additionally, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in treating, preventing, and/or diagnosing anemias associated with abnormal red blood cell architecture including but not limited to, hereditary spherocytosis, hereditary elliptocytosis, glucose-6-phosphate dehydrogenase deficiency, and sickle cell anemia.

[0652] The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in treating, preventing, and/or diagnosing hemoglobin abnormalities, (e.g., those associated with sickle cell anemia, hemoglobin C disease, hemoglobin S-C disease, and hemoglobin E disease). Additionally, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating thalassemias, including, but not limited to, major and minor forms of alpha-thalassemia and beta-thalassemia.

[0653] In another embodiment, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating bleeding disorders including, but not limited to, thrombocytopenia (e.g., idiopathic thrombocytopenic purpura, and thrombotic thrombocytopenic purpura), Von Willebrand's disease, hereditary platelet disorders (e.g., storage pool disease such as Chediak-Higashi and Hermansky-Pudlak syndromes, thromboxane A2 dysfunction, thrombasthenia, and Bernard-Soulier syndrome), hemolytic-uremic syndrome, hemophelias such as hemophelia A or Factor VII deficiency and Christmas disease or

Factor IX deficiency, Hereditary Hemorrhagic Telangiectasia, also known as Rendu-Osler-Weber syndrome, allergic purpura (Henoch Schonlein purpura) and disseminated intravascular coagulation.

[0654] The effect of the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention on the clotting time of blood may be monitored using any of the clotting tests known in the art including, but not limited to, whole blood partial thromboplastin time (PTT), the activated partial thromboplastin time (aPTT), the activated clotting time (ACT), the recalcified activated clotting time, or the Lee-White Clotting time.

[0655] Several diseases and a variety of drugs can cause platelet dysfunction. Thus, in a specific embodiment, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating acquired platelet dysfunction such as platelet dysfunction accompanying kidney failure, leukemia, multiple myeloma, cirrhosis of the liver, and systemic lupus erythematosus as well as platelet dysfunction associated with drug treatments, including treatment with aspirin, ticlopidine, nonsteroidal anti-inflammatory drugs (used for arthritis, pain, and sprains), and penicillin in high doses.

[0656] In another embodiment, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating diseases and disorders characterized by or associated with increased or decreased numbers of white blood cells. Leukopenia occurs when the number of white blood cells decreases below normal. Leukopenias include, but are not limited to, neutropenia and lymphocytopenia. An increase in the number of white blood cells compared to normal is known as leukocytosis. The body generates increased numbers of white blood cells during infection. Thus, leukocytosis may simply be a normal physiological parameter that reflects infection. Alternatively, leukocytosis may be an indicator of injury or other disease such as cancer. Leukocytoses, include but are not limited to, eosinophilia, and accumulations of macrophages. In specific embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating leukopenia. In other specific embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating leukocytosis.

[0657] Leukopenia may be a generalized decreased in all types of white blood cells, or may be a specific depletion of particular types of white blood cells. Thus, in specific embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating decreases in neutrophil numbers, known as neutropenia. Neutropenias that may be diagnosed, prognosed, prevented, and/or treated by the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, infantile genetic agranulocytosis, familial neutropenia, cyclic neutropenia, neutropenias resulting from or associated with dietary deficiencies (e.g., vitamin B 12 deficiency or folic acid deficiency), neutropenias resulting from or associated with drug treatments (e.g., antibiotic regimens such as penicillin treatment, sulfonamide treatment, anticoagulant treatment, anticonvulsant drugs, anti-thyroid drugs, and cancer chemotherapy), and neutropenias resulting from increased neutrophil destruction that may occur in association with some bacterial or viral infections, allergic disorders, autoimmune diseases, conditions in which an individual has an enlarged spleen (e.g., Felty syndrome, malaria and sarcoidosis), and some drug treatment regimens.

[0658] The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating lymphocytopenias (decreased numbers of B and/or T lymphocytes), including, but not limited to, lymphocytopenias resulting from or associated with stress, drug treatments (e.g., drug treatment with corticosteroids, cancer chemotherapies, and/or radiation therapies), AIDS infection and/or other diseases such as, for example, cancer, rheumatoid arthritis, systemic lupus erythematosus, chronic infections, some viral infections and/or hereditary disorders (e.g., DiGeorge syndrome, Wiskott-Aldrich Syndome, severe combined immunodeficiency, ataxia telangiectasia).

[0659] The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating diseases and disorders associated with macrophage numbers and/or macrophage function including, but not limited to, Gaucher's disease, Niemann-Pick disease, Letterer-Siwe disease and Hand-Schuller-Christian disease.

[0660] In another embodiment, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating diseases and disorders associated with eosinophil numbers and/or eosinophil function including, but not limited to, idiopathic hypereosinophilic syndrome, eosinophilia-myalgia syndrome, and Hand-Schuller-Christian disease.

[0661] In yet another embodiment, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating leukemias and lymphomas including, but not limited to, acute lymphocytic (lymphoblastic) leukemia (ALL), acute myeloid (myelocytic, myelogenous, myeloblastic, or myelomonocytic) leukemia, chronic lymphocytic leukemia (e.g., B cell leukemias, T cell leukemias, Sezary syndrome, and Hairy cell leukemia), chronic myelocytic (myeloid, myelogenous, or granulocytic) leukemia, Hodgkin's lymphoma, non-hodgkin's lymphoma, Burkitt's lymphoma, and mycosis fungoides.

[0662] In other embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating diseases and disorders of plasma cells including, but not limited to,

plasma cell dyscrasias, monoclonal gammopathies, monoclonal gammopathies of undetermined significance, multiple myeloma, macroglobulinemia, Waldenstrom's macroglobulinemia, cryoglobulinemia, and Raynaud's phenomenon.

[0663] In other embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in treating, preventing, and/or diagnosing myeloproliferative disorders, including but not limited to, polycythemia vera, relative polycythemia, secondary polycythemia, myelofibrosis, acute myelofibrosis, agnogenic myeloid metaplasia, thrombocythemia, (including both primary and secondary thrombocythemia) and chronic myelocytic leukemia.

[0664] In other embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful as a treatment prior to surgery, to increase blood cell production.

[0665] In other embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful as an agent to enhance the migration, phagocytosis, superoxide production, antibody dependent cellular cytotoxicity of neutrophils, eosinophils and macrophages.

[0666] In other embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful as an agent to increase the number of stem cells in circulation prior to stem cells pheresis. In another specific embodiment, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful as an agent to increase the number of stem cells in circulation prior to platelet pheresis.

[0667] In other embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful as an agent to increase cytokine production.

[0668] In other embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in preventing, diagnosing, and/or treating primary hematopoietic disorders.

Hyperproliferative Disorders

[0669] In certain embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention can be used to treat or detect hyperproliferative disorders, including neoplasms. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may proliferate other cells which can inhibit the hyperproliferative disorder.

[0670] For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

[0671] Examples of hyperproliferative disorders that can be treated or detected by fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to neoplasms located in the: colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvis, skin, soft tissue, spleen, thorax, and urogenital tract.

[0672] Similarly, other hyperproliferative disorders can also be treated or detected by fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention. Examples of such hyperproliferative disorders include, but are not limited to: Acute Childhood Lymphoblastic Leukemia, Acute Lymphoblastic Leukemia, Acute Lymphocytic Leukemia, Acute Myeloid Leukemia, Adrenocortical Carcinoma, Adult (Primary) Hepatocellular Cancer, Adult (Primary) Liver Cancer, Adult Acute Lymphocytic Leukemia, Adult Acute Myeloid Leukemia, Adult Hodgkin's Disease, Adult Hodgkin's Lymphoma, Adult Lymphocytic Leukemia, Adult Non-Hodgkin's Lymphoma, Adult Primary Liver Cancer, Adult Soft Tissue Sarcoma, AIDS-Related Lymphoma, AIDS-Related Malignancies, Anal Cancer, Astrocytoma, Bile Duct Cancer, Bladder Cancer, Bone Cancer, Brain Stem Glioma, Brain Tumors, Breast Cancer, Cancer of the Renal Pelvis and Ureter, Central Nervous System (Primary) Lymphoma, Central Nervous System Lymphoma, Cerebellar Astrocytoma, Cerebral Astrocytoma, Cervical Cancer, Childhood (Primary) Hepatocellular Cancer, Childhood (Primary) Liver Cancer, Childhood Acute Lymphoblastic Leukemia, Childhood Acute Myeloid Leukemia, Childhood Brain Stem Glioma, Childhood Cerebellar Astrocytoma, Childhood Cerebral Astrocytoma, Childhood Extracranial Germ Cell Tumors, Childhood Hodgkin's Disease, Childhood Hodgkin's Lymphoma, Childhood Hypothalamic and Visual Pathway Glioma, Childhood Lymphoblastic Leukemia, Childhood Medulloblastoma, Childhood Non-Hodgkin's Lymphoma, Childhood Pineal and Supratentorial Primitive Neuroectodermal Tumors, Childhood Primary Liver Cancer, Childhood Rhabdomyosarcoma, Childhood Soft Tissue Sarcoma, Childhood Visual Pathway and Hypothalamic Glioma, Chronic Lymphocytic Leukemia, Chronic Myelogenous Leukemia, Colon Cancer, Cutaneous T-Cell Lymphoma, Endocrine Pancreas Islet Cell Carcinoma, Endometrial Cancer, Ependymoma, Epithelial Cancer, Esophageal Cancer, Ewing's Sarcoma and Related Tumors, Exocrine Pancreatic Cancer, Extracranial Germ Cell Tumor, Extragonadal Germ Cell Tumor, Extrahepatic Bile Duct Cancer, Eye Cancer, Female Breast Cancer, Gaucher's Disease, Gallbladder Cancer, Gastric Cancer, Gastrointestinal Carcinoid Tumor, Gastrointestinal Tumors, Germ Cell Tumors, Gestational Trophoblastic Tumor, Hairy Cell Leukemia, Head and Neck Cancer, Hepatocellular Cancer, Hodgkin's Disease, Hodgkin's

Lymphoma, Hypergammaglobulinemia, Hypopharyngeal Cancer, Intestinal Cancers, Intraocular Melanoma, Islet Cell Carcinoma, Islet Cell Pancreatic Cancer, Kaposi's Sarcoma, Kidney Cancer, Laryngeal Cancer, Lip and Oral Cavity Cancer, Liver Cancer, Lung Cancer, Lymphoproliferative Disorders, Macroglobulinemia, Male Breast Cancer, Malignant Mesothelioma, Malignant Thymoma, Medulloblastoma, Melanoma, Mesothelioma, Metastatic Occult Primary Squamous Neck Cancer, Metastatic Primary Squamous Neck Cancer, Metastatic Squamous Neck Cancer, Multiple Myeloma, Multiple Myeloma/Plasma Cell Neoplasm, Myelodysplastic Syndrome, Myelogenous Leukemia, Myeloid Leukemia, Myeloproliferative Disorders, Nasal Cavity and Paranasal Sinus Cancer, Nasopharyngeal Cancer, Neuroblastoma, Non-Hodgkin's Lymphoma During Pregnancy, Nonmelanoma Skin Cancer, Non-Small Cell Lung Cancer, Occult Primary Metastatic Squamous Neck Cancer, Oropharyngeal Cancer, Osteo-/Malignant Fibrous Sarcoma, Osteosarcoma/Malignant Fibrous Histiocytoma, Osteosarcoma/Malignant Fibrous Histiocytoma of Bone, Ovarian Epithelial Cancer, Ovarian Germ Cell Tumor, Ovarian Low Malignant Potential Tumor, Pancreatic Cancer, Paraproteinemias, Purpura, Parathyroid Cancer, Penile Cancer, Pheochromocytoma, Pituitary Tumor, Plasma Cell Neoplasm/Multiple Myeloma, Primary Central Nervous System Lymphoma, Primary Liver Cancer, Prostate Cancer, Rectal Cancer, Renal Cell Cancer, Renal Pelvis and Ureter Cancer, Retinoblastoma, Rhabdomyosarcoma, Salivary Gland Cancer, Sarcoidosis Sarcomas, Sezary Syndrome, Skin Cancer, Small Cell Lung Cancer, Small Intestine Cancer, Soft Tissue Sarcoma, Squamous Neck Cancer, Stomach Cancer, Supratentorial Primitive Neuroectodermal and Pineal Tumors, T-Cell Lymphoma, Testicular Cancer, Thymoma, Thyroid Cancer, Transitional Cell Cancer of the Renal Pelvis and Ureter, Transitional Renal Pelvis and Ureter Cancer, Trophoblastic Tumors, Ureter and Renal Pelvis Cell Cancer, Urethral Cancer, Uterine Cancer, Uterine Sarcoma, Vaginal Cancer, Visual Pathway and Hypothalamic Glioma, Vulvar Cancer, Waldenstrom's Macroglobulinemia, Wilms' Tumor, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

[0673] In another preferred embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to diagnose, prognose, prevent, and/or treat premalignant conditions and to prevent progression to a neoplastic or malignant state, including but not limited to those disorders described above. Such uses are indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has occurred (for review of such abnormal growth conditions, see Robbins and Angell, 1976, Basic Pathology, 2d Ed., W. B. Saunders Co., Philadelphia, pp. 68-79.)

[0674] Hyperplasia is a form of controlled cell proliferation, involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. Hyperplastic disorders which can be diagnosed, prognosed, prevented, and/or treated with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, angiofollicular mediastinal lymph node hyperplasia, angiolymphoid hyperplasia with eosinophilia, atypical melanocytic hyperplasia, basal cell hyperplasia, benign giant lymph node hyperplasia, cementum hyperplasia, congenital adrenal hyperplasia, congenital sebaceous hyperplasia, cystic hyperplasia, cystic hyperplasia of the breast, denture hyperplasia, ductal hyperplasia, endometrial hyperplasia, fibromuscular hyperplasia, focal epithelial hyperplasia, gingival hyperplasia, *inflammatory* fibrous hyperplasia, *inflammatory* papillary hyperplasia, *intravascular* papillary endothelial hyperplasia, *nodular* hyperplasia of prostate, nodular regenerative hyperplasia, pseudoepitheliomatous hyperplasia, senile sebaceous hyperplasia, and verrucous hyperplasia.

[0675] Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplastic disorders which can be diagnosed, prognosed, prevented, and/or treated with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, agnogenic myeloid metaplasia, apocrine metaplasia, atypical metaplasia, autoparenchymatous metaplasia, connective tissue metaplasia, epithelial metaplasia, intestinal metaplasia, metaplastic anemia, metaplastic ossification, metaplastic polyps, myeloid metaplasia, primary myeloid metaplasia, secondary myeloid metaplasia, squamous metaplasia, squamous metaplasia of amnion, and symptomatic myeloid metaplasia.

[0676] Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation. Dysplastic disorders which can be diagnosed, prognosed, prevented, and/or treated with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, anhidrotic ectodermal dysplasia, anterofacial dysplasia, asphyxiating thoracic dysplasia, atriodigital dysplasia, bronchopulmonary dysplasia, cerebral dysplasia, cervical dysplasia, chondroectodermal dysplasia, cleidocranial dysplasia, congenital ectodermal dysplasia, craniodiaphysial dysplasia, craniocarpotarsal dysplasia, craniometaphysial dysplasia, dentin dysplasia, diaphysial dysplasia, ectodermal dysplasia, enamel dysplasia, encephalo-ophthalmic dysplasia, dysplasia epiphysialis hemimelica, dysplasia epiphysialis multiplex, dysplasia epiphysialis punctata, epithelial dysplasia, faciodigitogenital dysplasia, familial fibrous dysplasia of jaws, familial white folded dysplasia, fibromuscular dysplasia, fibrous dysplasia of bone, florid osseous dysplasia, hereditary renal-retinal dysplasia, hidrotic ectodermal dysplasia, hypohidrotic ectodermal dysplasia, lymphopenic thymic dysplasia, mammary dysplasia, mandibulofacial dysplasia, metaphysial dysplasia, Mondini dysplasia, monostotic fibrous dysplasia, mucoepithelial dysplasia, multiple epiphysial dysplasia, oculoauriculovertebral dysplasia, oculodentodigital dysplasia, oculovertebral dysplasia, odontogenic dysplasia, ophthalmomandibulomelic dysplasia,

perapical cemental dysplasia, polyostotic fibrous dysplasia, pseudoachondroplastic spondyloepiphysial dysplasia, retinal dysplasia, septo-optic dysplasia, spondyloepiphysial dysplasia, and ventriculoradial dysplasia.

[0677] Additional pre-neoplastic disorders which can be diagnosed, prognosed, prevented, and/or treated with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, benign dysproliferative disorders (e.g., benign tumors, fibrocystic conditions, tissue hypertrophy, intestinal polyps, colon polyps, and esophageal dysplasia), leukoplakia, keratoses, Bowen's disease, Farmer's Skin, solar cheilitis, and solar keratosis.

[0678] In another embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may be used to diagnose and/or prognose disorders associated with the tissue(s) in which the polypeptide of the invention is expressed.

[0679] In another embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention conjugated to a toxin or a radioactive isotope, as described herein, may be used to treat cancers and neoplasms, including, but not limited to, those described herein. In a further preferred embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention conjugated to a toxin or a radioactive isotope, as described herein, may be used to treat acute myelogenous leukemia.

[0680] Additionally, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may affect apoptosis, and therefore, would be useful in treating a number of diseases associated with increased cell survival or the inhibition of apoptosis. For example, diseases associated with increased cell survival or the inhibition of apoptosis that could be diagnosed, prognosed, prevented, and/or treated by polynucleotides, polypeptides, and/or agonists or antagonists of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection.

[0681] In preferred embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to inhibit growth, progression, and/or metastasis of cancers, in particular those listed above.

[0682] Additional diseases or conditions associated with increased cell survival that could be diagnosed, prognosed, prevented, and/or treated by fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

[0683] Diseases associated with increased apoptosis that could be diagnosed, prognosed, prevented, and/or treated by fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, retinitis pigmentosa, cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

[0684] Hyperproliferative diseases and/or disorders that could be diagnosed, prognosed, prevented, and/or treated by fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, include, but are not limited to, neoplasms located in the liver, abdomen, bone, breast, digestive system, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous system (central and peripheral), lymphatic system, pelvis, skin, soft tissue, spleen, thorax, and urogenital tract.

[0685] Similarly, other hyperproliferative disorders can also be diagnosed, prognosed, prevented, and/or treated by fusion proteins of the .

invention and/or polynucleotides encoding albumin fusion proteins of the invention. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstron's macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

[0686] Another preferred embodiment utilizes polynucleotides encoding albumin fusion proteins of the invention to inhibit aberrant cellular division, by gene therapy using the present invention, and/or protein fusions or fragments thereof.

[0687] Thus, the present invention provides a method for treating cell proliferative disorders by inserting into an abnormally proliferating cell a polynucleotide encoding an albumin fusion protein of the present invention, wherein said polynucleotide represses said expression.

[0688] Another embodiment of the present invention provides a method of treating cell-proliferative disorders in individuals comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells. In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said polynucleotides. In another preferred embodiment of the present invention, the DNA construct encoding the fusion protein of the present invention is inserted into cells to be treated utilizing a retrovirus, or more preferably an adenoviral vector (See G J. Nabel, et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference). In a most preferred embodiment, the viral vector is defective and will not transform non-proliferating cells, only proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present invention inserted into proliferating cells either alone, or in combination with or fused to other polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts upon the promoter upstream of said polynucleotides to induce expression of the encoded protein product. As such the beneficial therapeutic affect of the present invention may be expressly modulated (i.e. to increase, decrease, or inhibit expression of the present invention) based upon said external stimulus.

[0689] Polynucleotides of the present invention may be useful in repressing expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes " is intended the suppression of the transcription of the gene, the degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the destruction of the messenger RNA, the prevention of the post-translational modifications of the protein, the destruction of the protein, or the inhibition of the normal function of the protein.

[0690] For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in the art including, but not limited to transfection, electroporation, microinjection of cells, or in vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any other method described throughout the specification. The polynucleotide of the present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors (Gilboa, J. Virology 44:845 (1982); Hocke, Nature 320:275 (1986); Wilson, et al., Proc. Natl. Acad. Sci. U.S.A. 85:3014), vaccinia virus system (Chakraborty et al., Mol. Cell Biol. 5:3403 (1985) or other efficient DNA delivery systems (Yates et al., Nature 313:812 (1985)) known to those skilled in the art. These references are exemplary only and are hereby incorporated by reference. In order to specifically deliver or transfect cells which are abnormally proliferating and spare non-dividing cells, it is preferable to utilize a retrovirus, or adenoviral (as described in the art and elsewhere herein) delivery system known to those of skill in the art. Since host DNA replication is required for retroviral DNA to integrate and the retrovirus will be unable to self replicate due to the lack of the retrovirus genes needed for its life cycle. Utilizing such a retroviral delivery system for polynucleotides of the present invention will target said gene and constructs to abnormally proliferating cells and will spare the non-dividing normal cells.

[0691] The polynucleotides of the present invention may be delivered directly to cell proliferative disorder/disease sites in internal organs, body cavities and the like by use of imaging devices used to guide an injecting needle directly to the disease site. The polynucleotides of the present invention may also be administered to disease sites at the time of surgical intervention.

[0692] By "cell proliferative disease" is meant any human or animal disease or disorder, affecting any one or any combination of organs, cavities, or body parts, which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

[0693] Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of the treated cells. Moreover, it is possible to administer more than one of the polynucleotide of the present invention simultaneously to the same site. By "biologically inhibiting" is meant partial or total growth inhibition as well as decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of the present invention on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell cultures, or any other method known to one of ordinary skill in the art.

[0694] Moreover, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention of the present invention are useful in inhibiting the angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other polypeptides directly or indirectly, as described elsewhere herein. In a most preferred embodiment, said anti-angiogenesis effect may be achieved indirectly, for example, through the inhibition of hematopoietic, tumor-specific cells, such as tumor-associated macrophages (See Joseph IB, et al. J Natl Cancer Inst, 90(21):1648-53 (1998), which is hereby incorporated by reference).

[0695] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in inhibiting proliferative cells or tissues through the induction of apoptosis. These fusion proteins and/or polynucleotides may act either directly, or indirectly to induce apoptosis of proliferative cells and tissues, for example in the activation of a death-domain receptor, such as tumor necrosis factor (TNF) receptor-1, CD95 (Fas/APO-1), TNF-receptor-related apoptosis-mediated protein (TRAMP) and TNF-related apoptosis-inducing ligand (TRAIL) receptor-1 and -2 (See Schulze-Osthoff K, et.al., Eur J Biochem 254(3):439-59 (1998), which is hereby incorporated by reference). Moreover, in another preferred embodiment of the present invention, these fusion proteins and/or polynucleotides may induce apoptosis through other mechanisms, such as in the activation of other proteins which will activate apoptosis, or through stimulating the expression of these proteins, either alone or in combination with small molecule drugs or adjuvants, such as apoptonin, galectins, thioredoxins, anti-inflammatory proteins (See for example, Mutat Res 400(1-2):447-55 (1998), Med Hypotheses.50(5):423-33 (1998), Chem Biol Interact. Apr 24;111-112:23-34 (1998), J Mol Med.76(6):402-12 (1998), Int J Tissue React;20(1):3-15 (1998), which are all hereby incorporated by reference).

[0696] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are useful in inhibiting the metastasis of proliferative cells or tissues. Inhibition may occur as a direct result of administering these albumin fusion proteins and/or polynucleotides, or indirectly, such as activating the expression of proteins known to inhibit metastasis, for example alpha 4 integrins, (See, e.g., Curr Top Microbiol Immunol 1998;231:125-41, which is hereby incorporated by reference). Such therapeutic effects of the present invention may be achieved either alone, or in combination with small molecule drugs or adjuvants.

[0697] In another embodiment, the invention provides a method of delivering compositions containing the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention to targeted cells expressing the a polypeptide bound by, that binds to, or associates with an albumin fusion protein of the invention. Albumin fusion proteins of the invention may be associated with with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions.

[0698] Albumin fusion proteins of the invention are useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the albumin fusion proteins of the invention 'vaccinated' the immune response to respond to proliferative antigens and immunogens, or indirectly, such as in activating the expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

Renal Disorders

[0699] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may be used to treat, prevent, diagnose, and/or prognose disorders of the renal system. Renal disorders which can be diagnosed, prognosed, prevented, and/or treated with compositions of the invention include, but are not limited to, kidney failure, nephritis, blood vessel disorders of kidney, metabolic and congenital kidney disorders, urinary disorders of the kidney, autoimmune disorders, sclerosis and necrosis, electrolyte imbalance, and kidney cancers.

[0700] Kidney diseases which can be diagnosed, prognosed, prevented, and/or treated with compositions of the invention include, but are not limited to, acute kidney failure, chronic kidney failure, atheroembolic renal failure, end-stage renal disease, inflammatory diseases of the kidney (e.g., acute glomerulonephritis, postinfectious glomerulonephritis, rapidly progressive glomerulonephritis, nephrotic syndrome, membranous glomerulonephritis, familial nephrotic syndrome, membranoproliferative glomerulonephritis I and II, mesangial proliferative glomerulonephritis, chronic glomerulonephritis, acute tubulointerstitial nephritis, chronic tubulointerstitial nephritis, acute post-streptococcal glomerulonephritis (PSGN), pyelonephritis, lupus nephritis, chronic nephritis, interstitial nephritis, and post-streptococcal glomerulonephritis), blood vessel disorders of the kidneys (e.g., kidney infarction, atheroembolic kidney disease, cortical necrosis, malignant nephrosclerosis, renal vein thrombosis, renal underperfusion, renal retinopathy, renal ischemia-reperfusion, renal artery embolism, and renal artery stenosis), and kidney disorders resulting form urinary tract disease (e.g., pyelonephritis, hydronephrosis, urolithiasis (renal lithiasis, nephrolithiasis), reflux nephropathy, urinary tract infections, urinary retention, and acute or chronic unilateral obstructive uropathy.)

[0701] In addition, compositions of the invention can be used to diagnose, prognose, prevent, and/or treat metabolic and congenital disorders of the kidney (e.g., uremia, renal amyloidosis, renal osteodystrophy, renal tubular acidosis, renal glycosuria, nephrogenic diabetes insipidus, cystinuria, Fanconi's syndrome, renal fibrocystic osteosis (renal rickets), Hartnup disease, Bartter's syndrome, Liddle's syndrome, polycystic kidney disease, medullary cystic disease, medullary sponge kidney, Alport's syndrome, nail-patella syndrome, congenital nephrotic syndrome, CRUSH syndrome, horseshoe kidney, diabetic nephropathy, nephrogenic diabetes insipidus, analgesic nephropathy, kidney stones, and membranous nephropathy), and autoimmune disorders of the kidney (e.g., systemic lupus erythematosus (SLE), Goodpasture syndrome, IgA nephropathy, and IgM mesangial proliferative glomerulonephritis).

[0702] Compositions of the invention can also be used to diagnose, prognose, prevent, and/or treat sclerotic or necrotic disorders of the kidney (e.g., glomerulosclerosis, diabetic nephropathy, focal segmental glomerulosclerosis (FSGS), necrotizing glomerulonephritis, and renal papillary necrosis), cancers of the kidney (e.g., nephroma, hypernephroma, nephroblastoma, renal cell cancer, transitional cell cancer, renal adenocarcinoma, squamous cell cancer, and Wilm's tumor), and electrolyte imbalances (e.g., nephrocalcinosis, pyuria, edema, hydronephritis, proteinuria, hyponatremia, hypernatremia, hypokalemia, hyperkalemia, hypocalcemia, hypercalcemia, hypophosphatemia, and hyperphosphatemia).

[0703] Compositions of the invention may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppositorial solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. Compositions of the invention may be administered as part of a Therapeutic, described in more detail below. Methods of delivering polynucleotides of the invention are described in more detail herein.

Cardiovascular Disorders

[0704] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may be used to treat, prevent, diagnose, and/or prognose cardiovascular disorders, including, but not limited to, peripheral artery disease, such as limb ischemia.

[0705] Cardiovascular disorders include, but are not limited to, cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary stenosis, and Scimitar Syndrome. Congenital heart defects include, but are not limited to, aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid stenosis, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilogy of Fallot, ventricular heart septal defects.

[0706] Cardiovascular disorders also include, but are not limited to, heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy, post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease, ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

[0707] Arrhythmias include, but are not limited to, sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia, Torsades de Pointes, and ventricular tachycardia.

[0708] Heart valve diseases include, but are not limited to, aortic valve insufficiency, aortic valve stenosis, heart murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary stenosis, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid stenosis, tricuspid valve insufficiency, and tricuspid valve stenosis.

[0709] Myocardial diseases include, but are not limited to, alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Keams Syndrome, myocardial reperfusion injury, and myocarditis.

[0710] Myocardial ischemias include, but are not limited to, coronary disease, such as angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

[0711] Cardiovascular diseases also include vascular diseases such as aneurysms, angiodysplasia, angiomatosis, bacillary angiomatosis, Hippel-Lindau Disease, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular disorders, diabetic angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, stacia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous insufficiency.

[0712] Aneurysms include, but are not limited to, dissecting aneurysms, false aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

[0713] Arterial occlusive diseases include, but are not limited to, arteriosclerosis, intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

[0714] Cerebrovascular disorders include, but are not limited to, carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subarachnoid hemorrhage, cerebral infarction, cerebral ischemia (including transient), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.

[0715] Embolisms include, but are not limited to, air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat

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embolisms, pulmonary embolisms, and thromboembolisms. Thrombosis include, but are not limited to, coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, and thrombophlebitis.

[0716] Ischemic disorders include, but are not limited to, cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes, but is not limited to, aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

[0717] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppositorial solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. Methods of delivering polynucleotides are described in more detail herein.

Respiratory Disorders

[0718] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to treat, prevent, diagnose, and/or prognose diseases and/or disorders of the respiratory system.

[0719] Diseases and disorders of the respiratory system include, but are not limited to, nasal vestibulitis, nonallergic rhinitis (e.g., acute rhinitis, chronic rhinitis, atrophic rhinitis, vasomotor rhinitis), nasal polyps, and sinusitis, juvenile angiofibromas, cancer of the nose and juvenile papillomas, vocal cord polyps, nodules (singer's nodules), contact ulcers, vocal cord paralysis, laryngoceles, pharyngitis (e.g., viral and bacterial), tonsillitis, tonsillar cellulitis, parapharyngeal abscess, laryngitis, laryngoceles, and throat cancers (e.g., cancer of the nasopharynx, tonsil cancer, larynx cancer), lung cancer (e.g., squamous cell carcinoma, small cell (oat cell) carcinoma, large cell carcinoma, and adenocarcinoma), allergic disorders (eosinophilic pneumonia, hypersensitivity pneumonitis (e.g., extrinsic allergic alveolitis, allergic interstitial pneumonitis, organic dust pneumoconiosis, allergic bronchopulmonary aspergillosis, asthma, Wegener's granulomatosis (granulomatous vasculitis), Goodpasture's syndrome)), pneumonia (e.g., bacterial pneumonia (e.g., *Streptococcus pneumoniae* (pneumococcal pneumonia), *Staphylococcus aureus* (staphylococcal pneumonia), Gram-negative bacterial pneumonia (caused by, e.g., *Klebsiella* and *Pseudomas spp.*), *Mycoplasma pneumoniae* pneumonia, *Hemophilus influenzae* pneumonia, *Legionella pneumophila* (Legionnaires' disease), and *Chlamydia psittaci* (Psittacosis)), and viral pneumonia (e.g., influenza, chickenpox (varicella).

[0720] Additional diseases and disorders of the respiratory system include, but are not limited to bronchiolitis, polio (poliomyelitis), croup, respiratory syncytial viral infection, mumps, erythema infectiosum (fifth disease), roseola infantum, progressive rubella panencephalitis, german measles, and subacute sclerosing panencephalitis), fungal pneumonia (e.g., Histoplasmosis, Coccidioidomycosis, Blastomycosis, fungal infections in people with severely suppressed immune systems (e.g., cryptococcosis, caused by *Cryptococcus neoformans*; aspergillosis, caused by *Aspergillus spp.*; candidiasis, caused by *Candida*; and mucormycosis)), *Pneumocystis carinii* (pneumocystis pneumonia), atypical pneumonias (e.g., *Mycoplasma* and *Chlamydia* spp.), opportunistic infection pneumonia, nosocomial pneumonia, chemical pneumonitis, and aspiration pneumonia, pleural disorders (e.g., pleurisy, pleural effusion, and pneumothorax (e.g., simple spontaneous pneumothorax, complicated spontaneous pneumothorax, tension pneumothorax)), obstructive airway diseases (e.g., asthma, chronic obstructive pulmonary disease (COPD), emphysema, chronic or acute bronchitis), occupational lung diseases (e.g., silicosis, black lung (coal workers' pneumoconiosis), asbestosis, berylliosis, occupational asthma, byssinosis, and benign pneumoconioses), Infiltrative Lung Disease (e.g., pulmonary fibrosis (e.g., fibrosing alveolitis, usual interstitial pneumonia), idiopathic pulmonary fibrosis, desquamative interstitial pneumonia, lymphoid interstitial pneumonia, histiocytosis X (e.g., Letterer-Siwe disease, Hand-Schüller-Christian disease, eosinophilic granuloma), idiopathic pulmonary hemosiderosis, sarcoidosis and pulmonary alveolar proteinosis), Acute respiratory distress syndrome (also called, e.g., adult respiratory distress syndrome), edema, pulmonary embolism, bronchitis (e.g., viral, bacterial), bronchiectasis, atelectasis, lung abscess (caused by, e.g., *Staphylococcus aureus* or *Legionella pneumophila*), and cystic fibrosis.

Anti-Angiogenesis Activity

[0721] The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate. Rastinejad *et al.*, *Cell 56*:345-355 (1989). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization including solid tumor growth and metastases, arthritis, some types of eye disorders, and psoriasis. See, e.g., reviews by Moses *et al.*, *Biotech. 9*:630-634 (1991); Folkman *et al.*, *N. Engl. J. Med.*, *333*:1757-1763 (1995); Auerbach *et al.*, *J. Microvasc. Res. 29*:401-411 (1985); Folkman, Advances in Cancer Research, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Opthalmol. 94*:715-743 (1982); and Folkman *et al.*, Science *221*:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest

that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, *Science 235*:442-447 (1987).

[0723] The present invention provides for treatment of diseases or disorders associated with neovascularization by administration of fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman *et al.*, Medicine, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)).Thus, the present invention provides a method of treating an angiogenesis-related disease and/or disorder, comprising administering to an individual in need thereof a therapeutically effective amount of an albumin fusion protein of the invention and/or polynucleotides encoding an albumin fusion protein of the invention. For example, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be utilized in a variety of additional methods in order to therapeutically treat a cancer or tumor. Cancers which may be treated with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to solid tumors, including prostate, lung, breast, ovarian, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder, thyroid cancer; primary tumors and metastases; melanomas; glioblastoma; Kaposi's sarcoma; leiomyosarcoma; non- small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as leukemias. For example, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be delivered topically, in order to treat cancers such as skin cancer, head and neck tumors, breast tumors, and Kaposi's sarcoma.

[0723] Within yet other aspects, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be utilized to treat superficial forms of bladder cancer by, for example, intravesical administration. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be delivered directly into the tumor, or near the tumor site, via injection or a catheter. Of course, as the artisan of ordinary skill will appreciate, the appropriate mode of administration will vary according to the cancer to be treated. Other modes of delivery are discussed herein.

[0724] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in treating other disorders, besides cancers, which involve angiogenesis. These disorders include, but are not limited to: benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; atherosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uvietis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis.

[0725] For example, within one aspect of the present invention methods are provided for treating hypertrophic scars and keloids, comprising the step of administering albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention to a hypertrophic scar or keloid.

[0726] Within one embodiment of the present invention fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are directly injected into a hypertrophic scar or keloid, in order to prevent the progression of these lesions. This therapy is of particular value in the prophylactic treatment of conditions which are known to result in the development of hypertrophic scars and keloids (e.g., burns), and is preferably initiated after the proliferative phase has had time to progress (approximately 14 days after the initial injury), but before hypertrophic scar or keloid development. As noted above, the present invention also provides methods for treating neovascular diseases of the eye, including for example, corneal neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, retrolental fibroplasia and macular degeneration.

[0727] Moreover, Ocular disorders associated with neovascularization which can be treated with the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman *et al.*, *Am. J. Ophthal. 85*:704-710 (1978) and Gartner *et al.*, *Surv. Ophthal. 22*:291-312 (1978).

[0728] Thus, within one aspect of the present invention methods are provided for treating neovascular diseases of the eye such as corneal neovascularization (including corneal graft neovascularization), comprising the step of administering to a patient a therapeutically effective amount of a compound (e.g., fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention) to the cornea, such that the formation of blood vessels is inhibited. Briefly, the cornea is a tissue which normally lacks blood vessels. In certain pathological conditions however, capillaries may extend into the cornea from the pericorneal vascular plexus of the limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may become complete if the cornea completely opacifies. A wide variety of disorders can result in corneal neovascularization, including for example, corneal infections (e.g., trachoma, herpes simplex keratitis,

leishmaniasis and onchocerciasis), immunological processes (e.g., graft rejection and Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any cause), toxic and nutritional deficiency states, and as a complication of wearing contact lenses.

[0729] Within particularly preferred embodiments of the invention, may be prepared for topical administration in saline (combined with any of the preservatives and antimicrobial agents commonly used in ocular preparations), and administered in eyedrop form. The solution or suspension may be prepared in its pure form and administered several times daily. Alternatively, anti-angiogenic compositions, prepared as described above, may also be administered directly to the cornea. Within preferred embodiments, the anti-angiogenic composition is prepared with a muco-adhesive polymer which binds to cornea. Within further embodiments, the anti-angiogenic factors or anti-angiogenic compositions may be utilized as an adjunct to conventional steroid therapy. Topical therapy may also be useful prophylactically in corneal lesions which are known to have a high probability of inducing an angiogenic response (such as chemical burns). In these instances the treatment, likely in combination with steroids, may be instituted immediately to help prevent subsequent complications.

[0730] Within other embodiments, the compounds described above may be injected directly into the corneal stroma by an ophthalmologist under microscopic guidance. The preferred site of injection may vary with the morphology of the individual lesion, but the goal of the administration would be to place the composition at the advancing front of the vasculature (i.e., interspersed between the blood vessels and the normal cornea). In most cases this would involve perilimbic corneal injection to "protect" the cornea from the advancing blood vessels. This method may also be utilized shortly after a corneal insult in order to prophylactically prevent corneal neovascularization. In this situation the material could be injected in the perilimbic cornea interspersed between the corneal lesion and its undesired potential limbic blood supply. Such methods may also be utilized in a similar fashion to prevent capillary invasion of transplanted corneas. In a sustained-release form injections might only be required 2-3 times per year. A steroid could also be added to the injection solution to reduce inflammation resulting from the injection itself.

[0731] Within another aspect of the present invention, methods are provided for treating neovascular glaucoma, comprising the step of administering to a patient a therapeutically effective amount of an albumin fusion protein of the invention and/or polynucleotides encoding an albumin fusion protein of the invention to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the compound may be administered topically to the eye in order to treat early forms of neovascular glaucoma. Within other embodiments, the compound may be implanted by injection into the region of the anterior chamber angle. Within other embodiments, the compound may also be placed in any location such that the compound is continuously released into the aqueous humor. Within another aspect of the present invention, methods are provided for treating proliferative diabetic retinopathy, comprising the step of administering to a patient a therapeutically effective amount of an albumin fusion protein of the invention and/or polynucleotides encoding an albumin fusion protein of the invention to the eyes, such that the formation of blood vessels is inhibited.

[0732] Within particularly preferred embodiments of the invention, proliferative diabetic retinopathy may be treated by injection into the aqueous humor or the vitreous, in order to increase the local concentration of the polynucleotide, polypeptide, antagonist and/or agonist in the retina. Preferably, this treatment should be initiated prior to the acquisition of severe disease requiring photocoagulation.

[0733] Within another aspect of the present invention, methods are provided for treating retrolental fibroplasia, comprising the step of administering to a patient a therapeutically effective amount of an albumin fusion protein of the invention and/or polynucleotides encoding an albumin fusion protein of the invention to the eye, such that the formation of blood vessels is inhibited. The compound may be administered topically, via intravitreous injection and/or via intraocular implants.

[0734] Additionally, disorders which can be treated with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

[0735] Moreover, disorders and/or states, which can be treated, prevented, diagnosed, and/or prognosed with the the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention of the invention include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor metastasis, Kaposi's sarcoma, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, and uveitis, delayed wound healing, endometriosis, vasculogenesis, granulations, hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, Osler-Webber Syndrome, plaque neovascularization, telangiectasia, hemophiliac joints, angiofibroma fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth control agent by preventing vascularization required for embryo implantation controlling menstruation, diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (Rochele minalia quintosa), ulcers (Helicobacter pylori), Bartonellosis and bacillary angiomatosis.

[0736] In one aspect of the birth control method, an amount of the compound sufficient to block embryo implantation is administered before or after intercourse and fertilization have occurred, thus providing an effective method of birth control, possibly a "morning after" method. Albumin

fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may also be used in controlling menstruation or administered as either a peritoneal lavage fluid or for peritoneal implantation in the treatment of endometriosis.

[0737] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be incorporated into surgical sutures in order to prevent stitch granulomas.

[0738] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be utilized in a wide variety of surgical procedures. For example, within one aspect of the present invention a compositions (in the form of, for example, a spray or film) may be utilized to coat or spray an area prior to removal of a tumor, in order to isolate normal surrounding tissues from malignant tissue, and/or to prevent the spread of disease to surrounding tissues. Within other aspects of the present invention, compositions (e.g., in the form of a spray) may be delivered via endoscopic procedures in order to coat tumors, or inhibit angiogenesis in a desired locale. Within yet other aspects of the present invention, surgical meshes which have been coated with anti- angiogenic compositions of the present invention may be utilized in any procedure wherein a surgical mesh might be utilized. For example, within one embodiment of the invention a surgical mesh laden with an anti-angiogenic composition may be utilized during abdominal cancer resection surgery (e.g., subsequent to colon resection) in order to provide support to the structure, and to release an amount of the anti-angiogenic factor.

[0739] Within further aspects of the present invention, methods are provided for treating tumor excision sites, comprising administering albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention to the resection margins of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at the site is inhibited. Within one embodiment of the invention, the anti-angiogenic compound is administered directly to the tumor excision site (e.g., applied by swabbing, brushing or otherwise coating the resection margins of the tumor with the anti-angiogenic compound). Alternatively, the anti-angiogenic compounds may be incorporated into known surgical pastes prior to administration. Within particularly preferred embodiments of the invention, the anti-angiogenic compounds are applied after hepatic resections for malignancy, and after neurosurgical operations.

[0740] Within one aspect of the present invention, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be administered to the resection margin of a wide variety of tumors, including for example, breast, colon, brain and hepatic tumors. For example, within one embodiment of the invention, anti-angiogenic compounds may be administered to the site of a neurological tumor subsequent to excision, such that the formation of new blood vessels at the site are inhibited.

[0741] The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

[0742] Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

[0743] Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

[0744] Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

[0745] A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, (1992)); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, (1992)); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, (1987)); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, (1987)); Bisantrene (National Cancer Institute);

Lobenzarit disodium (N-(2)-carboxyphenyl-4- chloroanthranilic acid disodium or "CCA"; Takeuchi et al., Agents Actions 36:312-316, (1992)); Thalidomide; Angiostatic steroid; AGM-1470; carboxynaminolmidazole; and metalloproteinase inhibitors such as BB94.

Diseases at the Cellular Level

[0746] Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated, prevented, diagnosed, and/or prognosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection.

[0747] In preferred embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to inhibit growth, progression, and/or metasis of cancers, in particular those listed above.

[0748] Additional diseases or conditions associated with increased cell survival that could be treated or detected by fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

[0749] Diseases associated with increased apoptosis that could be treated, prevented, diagnosed, and/or prognosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, include, but are not limited to, AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestosis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Wound Healing and Epithelial Cell Proliferation

[0750] In accordance with yet a further aspect of the present invention, there is provided a process for utilizing fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound healing, and to stimulate hair follicle production and healing of dermal wounds. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associated with systemic treatment with steroids, radiation therapy and antineoplastic drugs and antimetabolites. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used to promote dermal reestablishment subsequent to dermal loss

[0751] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are types of grafts that fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepidermic grafts, avascular grafts, Blair-Brown grafts, bone graft, brephoplastic

grafts, cutis graft, delayed graft, dermic graft, epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omenpal graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, can be used to promote skin strength and to improve the appearance of aged skin.

[0752] It is believed that fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, will also produce changes in hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach, small intestine, and large intestine. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could promote proliferation of epithelial cells such as sebocytes, hair follicles, hepatocytes, type II pneumocytes, mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung, liver, and gastrointestinal tract. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may promote proliferation of endothelial cells, keratinocytes, and basal keratinocytes.

[0753] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could also be used to reduce the side effects of gut toxicity that result from radiation, chemotherapy treatments or viral infections. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may have a cytoprotective effect on the small intestine mucosa. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

[0754] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could also be used to treat gastric and duodenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large intestine, respectively. Thus, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used to promote the resurfacing of the mucosal surface to aid more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, is expected to have a significant effect on the production of mucus throughout the gastrointestinal tract and could be used to protect the intestinal mucosa from injurious substances that are ingested or following surgery. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used to treat diseases associate with the under expression.

[0755] Moreover, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used to prevent and heal damage to the lungs due to various pathological states. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, which could stimulate proliferation and differentiation and promote the repair of alveoli and brochiolar epithelium to prevent or treat acute or chronic lung damage. For example, emphysema, which results in the progressive loss of alveoli, and inhalation injuries, i.e., resulting from smoke inhalation and burns, that cause necrosis of the bronchiolar epithelium and alveoli could be effectively treated using polynucleotides or polypeptides, agonists or antagonists of the present invention. Also fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary displasia, in premature infants.

[0756] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could stimulate the proliferation and differentiation of hepatocytes and, thus, could be used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetracholoride and other hepatotoxins known in the art).

[0757] In addition, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used to maintain the islet function so as to alleviate, delay or prevent permanent manifestation of the disease. Also, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

Neural Activity and Neurological Diseases

[0758] The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used for the diagnosis and/or treatment of diseases, disorders, damage or injury of the brain and/or nervous system. Nervous system disorders that can be

treated with the compositions of the invention (e.g., fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention), include, but are not limited to, nervous system injuries, and diseases or disorders which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated in a patient (including human and non-human mammalian patients) according to the methods of the invention, include but are not limited to, the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems: (1) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia; (2) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries; (3) malignant lesions, in which a portion of the nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous system tissue; (4) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, or syphilis; (5) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to, degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis (ALS); (6) lesions associated with nutritional diseases or disorders, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including, but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration; (7) neurological lesions associated with systemic diseases including, but not limited to, diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis; (8) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and (9) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including, but not limited to, multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

[0759] In one embodiment, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to protect neural cells from the damaging effects of hypoxia. In a further preferred embodiment, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to protect neural cells from the damaging effects of cerebral hypoxia. According to this embodiment, the compositions of the invention are used to treat or prevent neural cell injury associated with cerebral hypoxia. In one non-exclusive aspect of this embodiment, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, are used to treat or prevent neural cell injury associated with cerebral ischemia. In another non-exclusive aspect of this embodiment, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat or prevent neural cell injury associated with cerebral infarction.

[0760] In another preferred embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat or prevent neural cell injury associated with a stroke. In a specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat or prevent cerebral neural cell injury associated with a stroke.

[0761] In another preferred embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat or prevent neural cell injury associated with a heart attack. In a specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat or prevent cerebral neural cell injury associated with a heart attack.

[0762] The compositions of the invention which are useful for treating or preventing a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of limitation, compositions of the invention which elicit any of the following effects may be useful according to the invention: (1) increased survival time of neurons in culture either in the presence or absence of hypoxia or hypoxic conditions; (2) increased sprouting of neurons in culture or *in vivo*; (3) increased production of a neuron-associated molecule in culture or *in vivo*, e.g., choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or (4) decreased symptoms of neuron dysfunction *in vivo*. Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may routinely be measured using a method set forth herein or otherwise known in the art, such as, for example, in Zhang *et al.*, *Proc Natl Acad Sci USA* 97:3637-42 (2000) or in Arakawa *et al.*, *J. Neurosci.*, *10*:3507-15 (1990); increased sprouting of neurons may be detected by methods known in the art, such as, for example, the methods set forth in Pestronk *et al.*, *Exp. Neurol.*, *70*:65-82 (1980), or Brown *et al.*, *Ann. Rev. Neurosci.*, *4*:17-42 (1981); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., using techniques known in the art and depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron conduction velocity, or functional disability.

[0763] In specific embodiments, motor neuron disorders that may be treated according to the invention include, but are not limited to, disorders such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well

as other components of the nervous system, as well as disorders that selectively affect neurons such as amyotrophic lateral sclerosis, and including, but not limited to, progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

[0764] Further, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may play a role in neuronal survival; synapse formation; conductance; neural differentiation, etc. Thus, compositions of the invention (including fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention) may be used to diagnose and/or treat or prevent diseases or disorders associated with these roles, including, but not limited to, learning and/or cognition disorders. The compositions of the invention may also be useful in the treatment or prevention of neurodegenerative disease states and/or behavioural disorders. Such neurodegenerative disease states and/or behavioral disorders include, but are not limited to, Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, compositions of the invention may also play a role in the treatment, prevention and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders.

[0765] Additionally, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may be useful in protecting neural cells from diseases, damage, disorders, or injury, associated with cerebrovascular disorders including, but not limited to, carotid artery diseases (e.g., carotid artery thrombosis, carotid stenosis, or Moyamoya Disease), cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformations, cerebral artery diseases, cerebral embolism and thrombosis (e.g., carotid artery thrombosis, sinus thrombosis, or Wallenberg's Syndrome), cerebral hemorrhage (e.g., epidural or subdural hematoma, or subarachnoid hemorrhage), cerebral infarction, cerebral ischemia (e.g., transient cerebral ischemia, Subclavian Steal Syndrome, or vertebrobasilar insufficiency), vascular dementia (e.g., multi-infarct), leukomalacia, periventricular, and vascular headache (e.g., cluster headache or migraines).

[0766] In accordance with yet a further aspect of the present invention, there is provided a process for utilizing fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, for therapeutic purposes, for example, to stimulate neurological cell proliferation and/or differentiation. Therefore, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to treat and/or detect neurologic diseases. Moreover, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, can be used as a marker or detector of a particular nervous system disease or disorder.

[0767] Examples of neurologic diseases which can be treated or detected with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, brain diseases, such as metabolic brain diseases which includes phenylketonuria such as maternal phenylketonuria, pyruvate carboxylase deficiency, pyruvate dehydrogenase complex deficiency, Wernicke's Encephalopathy, brain edema, brain neoplasms such as cerebellar neoplasms which include infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms, supratentorial neoplasms, canavan disease, cerebellar diseases such as cerebellar ataxia which include spinocerebellar degeneration such as ataxia telangiectasia, cerebellar dyssynergia, Friedreich's Ataxia, Machado-Joseph Disease, olivopontocerebellar atrophy, cerebellar neoplasms such as infratentorial neoplasms, diffuse cerebral sclerosis such as encephalitis periaxialis, globoid cell leukodystrophy, metachromatic leukodystrophy and subacute sclerosing panencephalitis.

[0768] Additional neurologic diseases which can be treated or detected with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include cerebrovascular disorders (such as carotid artery diseases which include carotid artery thrombosis, carotid stenosis and Moyamoya Disease), cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformations, cerebral artery diseases, cerebral embolism and thrombosis such as carotid artery thrombosis, sinus thrombosis and Wallenberg's Syndrome, cerebral hemorrhage such as epidural hematoma, subdural hematoma and subarachnoid hemorrhage, cerebral infarction, cerebral ischemia such as transient cerebral ischemia, Subclavian Steal Syndrome and vertebrobasilar insufficiency, vascular dementia such as multi-infarct dementia, periventricular leukomalacia, vascular headache such as cluster headache and migraine.

[0769] Additional neurologic diseases which can be treated or detected with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include dementia such as AIDS Dementia Complex, presenile dementia such as Alzheimer's Disease and Creutzfeldt-Jakob Syndrome, senile dementia such as Alzheimer's Disease and progressive supranuclear palsy, vascular dementia such as multi-infarct dementia, encephalitis which include encephalitis periaxialis, viral encephalitis such as epidemic encephalitis, Japanese Encephalitis, St. Louis Encephalitis, tick-borne encephalitis and West Nile Fever, acute disseminated encephalomyelitis, meningoencephalitis such as uveomeningoencephalitic syndrome, Postencephalitic Parkinson Disease and subacute sclerosing panencephalitis, encephalomalacia such as periventricular leukomalacia, epilepsy such as generalized epilepsy which includes infantile spasms, absence epilepsy, myoclonic epilepsy which includes MERRF Syndrome, tonic-clonic epilepsy, partial epilepsy such as complex partial epilepsy, frontal lobe epilepsy and temporal lobe epilepsy, post-traumatic epilepsy, status epilepticus such as Epilepsia Partialis Continua, and Hallervorden-Spatz Syndrome.

[0770] Additional neurologic diseases which can be treated or detected with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include hydrocephalus such as Dandy-Walker Syndrome and normal pressure hydrocephalus, hypothalamic

diseases such as hypothalamic neoplasms, cerebral malaria, narcolepsy which includes cataplexy, bulbar poliomyelitis, cerebri pseudotumor, Rett Syndrome, Reye's Syndrome, thalamic diseases, cerebral toxoplasmosis, intracranial tuberculoma and Zellweger Syndrome, central nervous system infections such as AIDS Dementia Complex, Brain Abscess, subdural empyema, encephalomyelitis such as Equine Encephalomyelitis, Venezuelan Equine Encephalomyelitis, Necrotizing Hemorrhagic Encephalomyelitis, Visna, and cerebral malaria.

[0771] Additional neurologic diseases which can be treated or detected with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include meningitis such as arachnoiditis, aseptic meningitis such as viral meningitis which includes lymphocytic choriomeningitis, Bacterial meningitis which includes Haemophilus Meningitis, Listeria Meningitis, Meningococcal Meningitis such as Waterhouse-Friderichsen Syndrome, Pneumococcal Meningitis and meningeal tuberculosis, fungal meningitis such as Cryptococcal Meningitis, subdural effusion, meningoencephalitis such as uvemeningoencephalitic syndrome, myelitis such as transverse myelitis, neurosyphilis such as tabes dorsalis, poliomyelitis which includes bulbar poliomyelitis and postpoliomyelitis syndrome, prion diseases (such as Creutzfeldt-Jakob Syndrome, Bovine Spongiform Encephalopathy, Gerstmann-Straussler Syndrome, Kuru, Scrapie), and cerebral toxoplasmosis.

[0772] Additional neurologic diseases which can be treated or detected with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include central nervous system neoplasms such as brain neoplasms that include cerebellar neoplasms such as infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms and supratentorial neoplasms, meningeal neoplasms, spinal cord neoplasms which include epidural neoplasms, demyelinating diseases such as Canavan Diseases, diffuse cerebral sclerosis which includes adrenoleukodystrophy, encephalitis periaxialis, globoid cell leukodystrophy, diffuse cerebral sclerosis such as metachromatic leukodystrophy, allergic encephalomyelitis, necrotizing hemorrhagic encephalomyelitis, progressive multifocal leukoencephalopathy, multiple sclerosis, central pontine myelinolysis, transverse myelitis, neuromyelitis optica, Scrapie, Swayback, Chronic Fatigue Syndrome, Visna, High Pressure Nervous Syndrome, Meningism, spinal cord diseases such as amyotonia congenita, amyotrophic lateral sclerosis, spinal muscular atrophy such as Werdnig-Hoffmann Disease, spinal cord compression, spinal cord neoplasms such as epidural neoplasms, syringomyelia, Tabes Dorsalis, Stiff-Man Syndrome, mental retardation such as Angelman Syndrome, Cri-du-Chat Syndrome, De Lange's Syndrome, Down Syndrome, Gangliosidoses such as gangliosidoses G(M1), Sandhoff Disease, Tay-Sachs Disease, Hartnup Disease, homocystinuria, Laurence-Moon- Biedl Syndrome, Lesch-Nyhan Syndrome, Maple Syrup Urine Disease, mucolipidosis such as fucosidosis, neuronal ceroid-lipofuscinosis, oculocerebrorenal syndrome, phenylketonuria such as maternal phenylketonuria, Prader-Willi Syndrome, Rett Syndrome, Rubinstein-Taybi Syndrome, Tuberous Sclerosis, WAGR Syndrome, nervous system abnormalities such as holoprosencephaly, neural tube defects such as anencephaly which includes hydrangencephaly, Arnold-Chairi Deformity, encephalocele, meningocele, meningomyelocele, spinal dysraphism such as spina bifida cystica and spina bifida occulta.

[0773] Additional neurologic diseases which can be treated or detected with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include hereditary motor and sensory neuropathies which include Charcot-Marie Disease, Hereditary optic atrophy, Refsum's Disease, hereditary spastic paraplegia, Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies such as Congenital Analgesia and Familial Dysautonomia, Neurologic manifestations (such as agnosia that include Gerstmann's Syndrome, Amnesia such as retrograde amnesia, apraxia, neurogenic bladder, cataplexy, communicative disorders such as hearing disorders that includes deafness, partial hearing loss, loudness recruitment and tinnitus, language disorders such as aphasia which include agraphia, anomia, broca aphasia, and Wernicke Aphasia, Dyslexia such as Acquired Dyslexia, language development disorders, speech disorders such as aphasia which includes anomia, broca aphasia and Wernicke Aphasia, articulation disorders, communicative disorders such as speech disorders which include dysarthria, echolalia, mutism and stuttering, voice disorders such as aphonia and hoarseness, decerebrate state, delirium, fasciculation, hallucinations, meningism, movement disorders such as angelman syndrome, ataxia, athetosis, chorea, dystonia, hypokinesia, muscle hypotonia, myoclonus, tic, tarticollis and tremor, muscle hypertonia such as muscle rigidity such as stiff-man syndrome, muscle spasticity, paralysis such as facial paralysis which includes Herpes Zoster Oticus, Gastroparesis, Hemiplegia, ophthalmoplegia such as diplopia, Duane's Syndrome, Horner's Syndrome, Chronic progressive external ophthalmoplegia such as Kearns Syndrome, Bulbar Paralysis, Tropical Spastic Paraparesis, Paraplegia such as Brown-Sequard Syndrome, quadriplegia, respiratory paralysis and vocal cord paralysis, paresis, phantom limb, taste disorders such as ageusia and dysgeusia, vision disorders such as amblyopia, blindness, color vision defects, diplopia, hemianopsia, scotoma and subnormal vision, sleep disorders such as hypersomnia which includes Kleine-Levin Syndrome, insomnia, and somnambulism, spasm such as trismus, unconsciousness such as coma, persistent vegetative state and syncope and vertigo, neuromuscular diseases such as amyotonia congenita, amyotrophic lateral sclerosis, Lambert-Eaton Myasthenic Syndrome, motor neuron disease, muscular atrophy such as spinal muscular atrophy, Charcot-Marie Disease and Werdnig-Hoffmann Disease, Postpoliomyelitis Syndrome, Muscular Dystrophy, Myasthenia Gravis, Myotonia Atrophica, Myotonia Confenita, Nemaline Myopathy, Familial Periodic Paralysis, Multiplex Paramyoclonus, Tropical Spastic Paraparesis and Stiff-Man Syndrome, peripheral nervous system diseases such as acrodynia, amyloid neuropathies, autonomic nervous system diseases such as Adie's Syndrome, Barre-Lieou Syndrome, Familial Dysautonomia, Horner's Syndrome, Reflex Sympathetic Dystrophy and Shy-Drager Syndrome, Cranial Nerve Diseases such as Acoustic Nerve Diseases such as Acoustic Neuroma which includes Neurofibromatosis 2, Facial Nerve Diseases such as Facial Neuralgia,Melkersson-Rosenthal Syndrome, ocular motility disorders which includes amblyopia, nystagmus, oculomotor nerve paralysis, ophthalmoplegia such as Duane's Syndrome, Horner's

Syndrome, Chronic Progressive External Ophthalmoplegia which includes Kearns Syndrome, Strabismus such as Esotropia and Exotropia, Oculomotor Nerve Paralysis, Optic Nerve Diseases such as Optic Atrophy which includes Hereditary Optic Atrophy, Optic Disk Drusen, Optic Neuritis such as Neuromyelitis Optica, Papilledema, Trigeminal Neuralgia, Vocal Cord Paralysis, Demyelinating Diseases such as Neuromyelitis Optica and Swayback, and Diabetic neuropathies such as diabetic foot.

[0774] Additional neurologic diseases which can be treated or detected with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include nerve compression syndromes such as carpal tunnel syndrome, tarsal tunnel syndrome, thoracic outlet syndrome such as cervical rib syndrome, ulnar nerve compression syndrome, neuralgia such as causalgia, cervico-brachial neuralgia, facial neuralgia and trigeminal neuralgia, neuritis such as experimental allergic neuritis, optic neuritis, polyneuritis, polyradiculoneuritis and radiculitis such as polyradiculitis, hereditary motor and sensory neuropathies such as Charcot-Marie Disease, Hereditary Optic Atrophy, Refsum's Disease, Hereditary Spastic Paraplegia and Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies which include Congenital Analgesia and Familial Dysautonomia, POEMS Syndrome, Sciatica, Gustatory Sweating and Tetany).

Endocrine Disorders

[0775] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may be used to treat, prevent, diagnose, and/or prognose disorders and/or diseases related to hormone imbalance, and/or disorders or diseases of the endocrine system.

[0776] Hormones secreted by the glands of the endocrine system control physical growth, sexual function, metabolism, and other functions. Disorders may be classified in two ways: disturbances in the production of hormones, and the inability of tissues to respond to hormones. The etiology of these hormone imbalance or endocrine system diseases, disorders or conditions may be genetic, somatic, such as cancer and some autoimmune diseases, acquired (e.g., by chemotherapy, injury or toxins), or infectious. Moreover, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention can be used as a marker or detector of a particular disease or disorder related to the endocrine system and/or hormone imbalance.

[0777] Endocrine system and/or hormone imbalance and/or diseases encompass disorders of uterine motility including, but not limited to: complications with pregnancy and labor (e.g., pre-term labor, post-term pregnancy, spontaneous abortion, and slow or stopped labor); and disorders and/or diseases of the menstrual cycle (e.g., dysmenorrhea and endometriosis).

[0778] Endocrine system and/or hormone imbalance disorders and/or diseases include disorders and/or diseases of the pancreas, such as, for example, diabetes mellitus, diabetes insipidus, congenital pancreatic agenesis, pheochromocytoma--islet cell tumor syndrome; disorders and/or diseases of the adrenal glands such as, for example, Addison's Disease, corticosteroid deficiency, virilizing disease, hirsutism, Cushing's Syndrome, hyperaldosteronism, pheochromocytoma; disorders and/or diseases of the pituitary gland, such as, for example, hyperpituitarism, hypopituitarism, pituitary dwarfism, pituitary adenoma, panhypopituitarism, acromegaly, gigantism; disorders and/or diseases of the thyroid, including but not limited to, hyperthyroidism, hypothyroidism, Plummer's disease, Graves' disease (toxic diffuse goiter), toxic nodular goiter, thyroiditis (Hashimoto's thyroiditis, subacute granulomatous thyroiditis, and silent lymphocytic thyroiditis), Pendred's syndrome, myxedema, cretinism, thyrotoxicosis, thyroid hormone coupling defect, thymic aplasia, Hurthle cell tumours of the thyroid, thyroid cancer, thyroid carcinoma, Medullary thyroid carcinoma; disorders and/or diseases of the parathyroid, such as, for example, hyperparathyroidism, hypoparathyroidism; disorders and/or diseases of the hypothalamus.

[0779] In addition, endocrine system and/or hormone imbalance disorders and/or diseases may also include disorders and/or diseases of the testes or ovaries, including cancer. Other disorders and/or diseases of the testes or ovaries further include, for example, ovarian cancer, polycystic ovary syndrome, Klinefelter's syndrome, vanishing testes syndrome (bilateral anorchia), congenital absence of Leydig's cells, cryptorchidism, Noonan's syndrome, myotonic dystrophy, capillary haemangioma of the testis (benign), neoplasias of the testis and neo-testis.

[0780] Moreover, endocrine system and/or hormone imbalance disorders and/or diseases may also include disorders and/or diseases such as, for example, polyglandular deficiency syndromes, pheochromocytoma, neuroblastoma, multiple Endocrine neoplasia, and disorders and/or cancers of endocrine tissues.

[0781] In another embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may be used to diagnose, prognose, prevent, and/or treat endocrine diseases and/or disorders associated with the tissue(s) in which the Therapeutic protein corresponding to the Therapeutic protein portion of the albumin protein of the invention is expressed.

Reproductive System Disorders

[0782] The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used for the diagnosis, treatment, or prevention of diseases and/or disorders of the reproductive system. Reproductive system disorders that can be treated by the compositions of the invention, include, but are not limited to, reproductive system injuries, infections, neoplastic disorders, congenital defects, and diseases or disorders which result in infertility, complications with pregnancy, labor, or parturition, and postpartum difficulties.

[0783] Reproductive system disorders and/or diseases include diseases and/or disorders of the testes, including testicular atrophy, testicular feminization, cryptorchism (unilateral and bilateral), anorchia, ectopic testis, epididymitis and orchitis (typically resulting from infections such as, for example, gonorrhea, mumps, tuberculosis, and syphilis), testicular torsion, vasitis nodosa, germ cell tumors (e.g., seminomas, embryonal cell

carcinomas, teratocarcinomas, choriocarcinomas, yolk sac tumors, and teratomas), stromal tumors (e.g., Leydig cell tumors), hydrocele, hematocele, varicocele, spermatocele, inguinal hernia, and disorders of sperm production (e.g., immotile cilia syndrome, aspermia, asthenozoospermia, azoospermia, oligospermia, and teratozoospermia).

[0784] Reproductive system disorders also include disorders of the prostate gland, such as acute non-bacterial prostatitis, chronic non-bacterial prostatitis, acute bacterial prostatitis, chronic bacterial prostatitis, prostatodynia, prostatosis, granulomatous prostatitis, malacoplakia, benign prostatic hypertrophy or hyperplasia, and prostate neoplastic disorders, including adenocarcinomas, transitional cell carcinomas, ductal carcinomas, and squamous cell carcinomas.

[0785] Additionally, the compositions of the invention may be useful in the diagnosis, treatment, and/or prevention of disorders or diseases of the penis and urethra, including inflammatory disorders, such as balanoposthitis, balanitis xerotica obliterans, phimosis, paraphimosis, syphilis, herpes simplex virus, gonorrhea, non-gonococcal urethritis, chlamydia, mycoplasma, trichomonas, HIV, AIDS, Reiter's syndrome, condyloma acuminatum, condyloma latum, and pearly penile papules; urethral abnormalities, such as hypospadias, epispadias, and phimosis; premalignant lesions, including Erythroplasia of Queyrat, Bowen's disease, Bowenoid papiosis, giant condyloma of Buschke-Lowenstein, and verrucous carcinoma; penile cancers, including squamous cell carcinomas, carcinoma in situ, verrucous carcinoma, and disseminated penile carcinoma; urethral neoplastic disorders, including penile urethral carcinoma, bulbomembranous urethral carcinoma, and prostatic urethral carcinoma; and erectile disorders, such as priapism, Peyronie's disease, erectile dysfunction, and impotence.

[0786] Moreover, diseases and/or disorders of the vas deferens include vasculitis and CBAVD (congenital bilateral absence of the vas deferens); additionally, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used in the diagnosis, treatment, and/or prevention of diseases and/or disorders of the seminal vesicles, including hydatid disease, congenital chloride diarrhea, and polycystic kidney disease.

[0787] Other disorders and/or diseases of the male reproductive system include, for example, Klinefelter's syndrome, Young's syndrome, premature ejaculation, diabetes mellitus, cystic fibrosis, Kartagener's syndrome, high fever, multiple sclerosis, and gynecomastia.

[0788] Further, the polynucleotides, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used in the diagnosis, treatment, and/or prevention of diseases and/or disorders of the vagina and vulva, including bacterial vaginosis, candida vaginitis, herpes simplex virus, chancroid, granuloma inguinale, lymphogranuloma venereum, scabies, human papillomavirus, vaginal trauma, vulvar trauma, adenosis, chlamydia vaginitis, gonorrhea, trichomonas vaginitis, condyloma acuminatum, syphilis, molluscum contagiosum, atrophic vaginitis, Paget's disease, lichen sclerosus, lichen planus, vulvodynia, toxic shock syndrome, vaginismus, vulvovaginitis, vulvar vestibulitis, and neoplastic disorders, such as squamous cell hyperplasia, clear cell carcinoma, basal cell carcinoma, melanomas, cancer of Bartholin's gland, and vulvar intraepithelial neoplasia.

[0789] Disorders and/or diseases of the uterus include dysmenorrhea, retroverted uterus, endometriosis, fibroids, adenomyosis, anovulatory bleeding, amenorrhea, Cushing's syndrome, hydatidiform moles, Asherman's syndrome, premature menopause, precocious puberty, uterine polyps, dysfunctional uterine bleeding (e.g., due to aberrant hormonal signals), and neoplastic disorders, such as adenocarcinomas, leiomyosarcomas, and sarcomas. Additionally, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful as a marker or detector of, as well as in the diagnosis, treatment, and/or prevention of congenital uterine abnormalities, such as bicornuate uterus, septate uterus, simple unicornuate uterus, unicornuate uterus with a noncavitary rudimentary horn, unicornuate uterus with a non-communicating cavitary rudimentary horn, unicornuate uterus with a communicating cavitary horn, arcuate uterus, uterine didelfus, and T-shaped uterus.

[0790] Ovarian diseases and/or disorders include anovulation, polycystic ovary syndrome (Stein-Leventhal syndrome), ovarian cysts, ovarian hypofunction, ovarian insensitivity to gonadotropins, ovarian overproduction of androgens, right ovarian vein syndrome, amenorrhea, hirsutism, and ovarian cancer (including, but not limited to, primary and secondary cancerous growth, Sertoli-Leydig tumors, endometrioid carcinoma of the ovary, ovarian papillary serous adenocarcinoma, ovarian mucinous adenocarcinoma, and Ovarian Krukenberg tumors).

[0791] Cervical diseases and/or disorders include cervicitis, chronic cervicitis, mucopurulent cervicitis, cervical dysplasia, cervical polyps, Nabothian cysts, cervical erosion, cervical incompetence, and cervical neoplasms (including, for example, cervical carcinoma, squamous metaplasia, squamous cell carcinoma, adenosquamous cell neoplasia, and columnar cell neoplasia).

[0792] Additionally, diseases and/or disorders of the reproductive system include disorders and/or diseases of pregnancy, including miscarriage and stillbirth, such as early abortion, late abortion, spontaneous abortion, induced abortion, therapeutic abortion, threatened abortion, missed abortion, incomplete abortion, complete abortion, habitual abortion, missed abortion, and septic abortion; ectopic pregnancy, anemia, Rh incompatibility, vaginal bleeding during pregnancy, gestational diabetes, intrauterine growth retardation, polyhydramnios, HELLP syndrome, abruptio placentae, placenta previa, hyperemesis, preeclampsia, eclampsia, herpes gestationis, and urticaria of pregnancy. Additionally, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used in the diagnosis, treatment, and/or prevention of diseases that can complicate pregnancy, including heart disease, heart failure, rheumatic heart disease, congenital heart disease, mitral valve prolapse, high blood pressure, anemia, kidney disease, infectious disease (e.g., rubella, cytomegalovirus, toxoplasmosis, infectious

hepatitis, enterovirus, HIV, AIDS, and genital herpes), diabetes mellitus, Graves' disease, thyroiditis, hypothyroidism, Hashimoto's thyroiditis, chronic active hepatitis, cirrhosis of the liver, primary biliary cirrhosis, asthma, systemic lupus erycnatosis, rheumatoid arthritis, myasthenia gravis, idiopathic thrombocytopenic purpura, appendicitis, ovarian cysts, gallbladder disorders,and obstruction of the intestine.

[0793] Complications associated with labor and parturition include premature rupture of the membranes, pre-term labor, post-term pregnancy, postmaturity, labor that progresses too slowly, fetal distress (e.g., abnormal heart rate (fetal or maternal), breathing problems, and abnormal fetal position), shoulder dystocia, prolapsed umbilical cord, amniotic fluid embolism, and aberrant uterine bleeding.

[0794] Further, diseases and/or disorders of the postdelivery period, including endometritis, myometritis, parametritis, peritonitis, pelvic thrombophlebitis, pulmonary embolism, endotoxemia, pyelonephritis, saphenous thrombophlebitis, mastitis, cystitis, postpartum hemorrhage, and inverted uterus.

[0795] Other disorders and/or diseases of the female reproductive system that may be diagnosed, treated, and/or prevented by the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, for example, Turner's syndrome, pseudohermaphroditism, premenstrual syndrome, pelvic inflammatory disease, pelvic congestion (vascular engorgement), frigidity, anorgasmia, dyspareunia, ruptured fallopian tube, and Mittelschmerz.

Infectious Disease

[0796] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

[0797] Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention. Examples of viruses, include, but are not limited to Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, can be used to treat or detect any of these symptoms or diseases. In specific embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat AIDS.

[0798] Similarly, bacterial and fungal agents that can cause disease or symptoms and that can be treated or detected by albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but not limited to, the following Gram-Negative and Gram-positive bacteria, bacterial families, and fungi: Actinomyces (e.g., Norcardia), Acinetobacter, *Cryptococcus neoformans*, Aspergillus, Bacillaceae (e.g., *Bacillus anthracis*), Bacteroides (e.g., *Bacteroides fragilis*), Blastomycosis, Bordetella, Borrelia (e.g., *Borrelia burgdorferi*), Brucella, Candidia, Campylobacter, Chlamydia, Clostridium (e.g., *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Clostridium tetani*), Coccidioides, Corynebacterium (e.g., *Corynebacterium diphtheriae*), Cryptococcus, Dermatocycoses, *E. coli* (e.g., Enterotoxigenic *E. coli* and Enterohemorrhagic *E. coli*), Enterobacter (e.g. *Enterobacter aerogenes*), Enterobacteriaceae (Klebsiella, Salmonella (e.g., *Salmonella typhi*, *Salmonella enteritidis*, *Salmonella typhi*), Serratia, Yersinia, Shigella), Erysipelothrix, Haemophilus (e.g., *Haemophilus influenza* type B), Helicobacter, Legionella (e.g., *Legionella pneumophila*), Leptospira, Listeria (e.g., *Listeria monocytogenes*), Mycoplasma, Mycobacterium (e.g., *Mycobacterium leprae and Mycobacterium tuberculosis*), Vibrio (e.g., *Vibrio cholerae*), Neisseriaceae (e.g., *Neisseria gonorrhea*, *Neisseria meningitidis*), Pasteurellaceae, Proteus, Pseudomonas (e.g., *Pseudomonas aeruginosa*), Rickettsiaceae, Spirochetes (e.g., Treponema spp., Leptospira spp., Borrelia spp.), Shigella spp., Staphylococcus (e.g., *Staphylococcus aureus*), Meningiococcus, Pneumococcus and Streptococcus (e.g., *Streptococcus pneumoniae* and Groups A, B, and C Streptococci), and Ureaplasmas. These bacterial, parasitic, and fungal families can cause diseases or symptoms, including, but not limited to: antibiotic-resistant infections, bacteremia, endocarditis, septicemia, eye

infections (e.g., conjunctivitis), uveitis, tuberculosis, gingivitis, bacterial diarrhea, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, dental caries, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, dysentery, paratyphoid fever, food poisoning, Legionella disease, chronic and acute inflammation, erythema, yeast infections, typhoid, pneumonia, gonorrhea, meningitis (e.g., mengitis types A and B), chlamydia, syphillis, diphtheria, leprosy, brucellosis, peptic ulcers, anthrax, spontaneous abortions, birth defects, pneumonia, lung infections, ear infections, deafness, blindness, lethargy, malaise, vomiting, chronic diarrhea, Crohn's disease, colitis, vaginosis, sterility, pelvic inflammatory diseases, candidiasis, paratuberculosis, tuberculosis, lupus, botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections, nosocomial infections. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, can be used to treat or detect any of these symptoms or diseases. In specific embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat: tetanus, diptheria, botulism, and/or meningitis type B.

[0799] Moreover, parasitic agents causing disease or symptoms that can be treated, prevented, and/or diagnosed by fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiss, Helminthiasis, Leishmaniasis, Schistisoma, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., *Plasmodium vivax*, *Plasmodium falciparium*, *Plasmodium malariae* and *Plasmodium ovale*). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, can be used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat, prevent, and/or diagnose malaria.

[0800] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention could either be by administering an effective amount of an albumin fusion protein of the invention to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

Regeneration

[0801] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997)). The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

[0802] Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

[0803] Moreover, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

[0804] Similarly, nerve and brain tissue could also be regenerated by using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stoke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention.

Gastrointestinal Disorders

[0805] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may be used to treat, prevent, diagnose, and/or prognose gastrointestinal disorders, including inflammatory diseases and/or conditions, infections, cancers (e.g., intestinal

neoplasms (carcinoid tumor of the small intestine, non-Hodgkin's lymphoma of the small intestine, small bowel lymphoma)), and ulcers, such as peptic ulcers.

[0806] Gastrointestinal disorders include dysphagia, odynophagia, inflammation of the esophagus, peptic esophagitis, gastric reflux, submucosal fibrosis and stricturing, Mallory-Weiss lesions, leiomyomas, lipomas, epidermal cancers, adenocarcinomas, gastric retention disorders, gastroenteritis, gastric atrophy, gastric/stomach cancers, polyps of the stomach, autoimmune disorders such as pernicious anemia, pyloric stenosis, gastritis (bacterial, viral, eosinophilic, stress-induced, chronic erosive, atrophic, plasma cell, and Ménétrier's), and peritoneal diseases (e.g., chyloperitoneum, hemoperitoneum, mesenteric cyst, mesenteric lymphadenitis, mesenteric vascular occlusion, panniculitis, neoplasms, peritonitis, pneumoperitoneum, subphrenic abscess,).

[0807] Gastrointestinal disorders also include disorders associated with the small intestine, such as malabsorption syndromes, distension, irritable bowel syndrome, sugar intolerance, celiac disease, duodenal ulcers, duodenitis, tropical sprue, Whipple's disease, intestinal lymphangiectasia, Crohn's disease, appendicitis, obstructions of the ileum, Meckel's diverticulum, multiple diverticula, failure of complete rotation of the small and large intestine, lymphoma, and bacterial and parasitic diseases (such as Traveler's diarrhea, typhoid and paratyphoid, cholera, infection by Roundworms (*Ascariasis lumbricoides*), Hookworms (*Ancylostoma duodenale*), Threadworms (*Enterobius vermicularis*), Tapeworms (*Taenia saginata, Echinococcus granulosus, Diphyllobothrium spp.*, and *T. solium*).

[0808] Liver diseases and/or disorders include intrahepatic cholestasis (alagille syndrome, biliary liver cirrhosis), fatty liver (alcoholic fatty liver, reye syndrome), hepatic vein thrombosis, hepatolenticular degeneration, hepatomegaly, hepatopulmonary syndrome, hepatorenal syndrome, portal hypertension (esophageal and gastric varices), liver abscess (amebic liver abscess), liver cirrhosis (alcoholic, biliary and experimental), alcoholic liver diseases (fatty liver, hepatitis, cirrhosis), parasitic (hepatic echinococcosis, fascioliasis, amebic liver abscess), jaundice (hemolytic, hepatocellular, and cholestatic), cholestasis, portal hypertension, liver enlargement, ascites, hepatitis (alcoholic hepatitis, animal hepatitis, chronic hepatitis (autoimmune, hepatitis B, hepatitis C, hepatitis D, drug induced), toxic hepatitis, viral human hepatitis (hepatitis A, hepatitis B, hepatitis C, hepatitis D, hepatitis E), Wilson's disease, granulomatous hepatitis, secondary biliary cirrhosis, hepatic encephalopathy, portal hypertension, varices, hepatic encephalopathy, primary biliary cirrhosis, primary sclerosing cholangitis, hepatocellular adenoma, hemangiomas, bile stones, liver failure (hepatic encephalopathy, acute liver failure), and liver neoplasms (angiomyolipoma, calcified liver metastases, cystic liver metastases, epithelial tumors, fibrolamellar hepatocarcinoma, focal nodular hyperplasia, hepatic adenoma, hepatobiliary cystadenoma, hepatoblastoma, hepatocellular carcinoma, hepatoma, liver cancer, liver hemangioendothelioma, mesenchymal hamartoma, mesenchymal tumors of liver, nodular regenerative hyperplasia, benign liver tumors (Hepatic cysts [Simple cysts, Polycystic liver disease, Hepatobiliary cystadenoma, Choledochal cyst], Mesenchymal tumors [Mesenchymal hamartoma, Infantile hemangioendothelioma, Hemangioma, Peliosis hepatis, Lipomas, Inflammatory pseudotumor, Miscellaneous], Epithelial tumors [Bile duct epithelium (Bile duct hamartoma, Bile duct adenoma), Hepatocyte (Adenoma, Focal nodular hyperplasia, Nodular regenerative hyperplasia)], malignant liver tumors [hepatocellular, hepatoblastoma, hepatocellular carcinoma, cholangiocellular, cholangiocarcinoma, cystadenocarcinoma, tumors of blood vessels, angiosarcoma, Karposi's sarcoma, hemangioendothelioma, other tumors, embryonal sarcoma, fibrosarcoma, leiomyosarcoma, rhabdomyosarcoma, carcinosarcoma, teratoma, carcinoid, squamous carcinoma, primary lymphoma]), peliosis hepatis, erythrohepatic porphyria, hepatic porphyria (acute intermittent porphyria, porphyria cutanea tarda), Zellweger syndrome).

[0809] Pancreatic diseases and/or disorders include acute pancreatitis, chronic pancreatitis (acute necrotizing pancreatitis, alcoholic pancreatitis), neoplasms (adenocarcinoma of the pancreas, cystadenocarcinoma, insulinoma, gastrinoma, and glucagonoma, cystic neoplasms, islet-cell tumors, pancreoblastoma), and other pancreatic diseases (e.g., cystic fibrosis, cyst (pancreatic pseudocyst, pancreatic fistula, insufficiency)).

[0810] Gallbladder diseases include gallstones (cholelithiasis and choledocholithiasis), postcholecystectomy syndrome, diverticulosis of the gallbladder, acute cholecystitis, chronic cholecystitis, bile duct tumors, and mucocele.

[0811] Diseases and/or disorders of the large intestine include antibiotic-associated colitis, diverticulitis, ulcerative colitis, acquired megacolon, abscesses, fungal and bacterial infections, anorectal disorders (e.g., fissures, hemorrhoids), colonic diseases (colitis, colonic neoplasms [colon cancer, adenomatous colon polyps (e.g., villous adenoma), colon carcinoma, colorectal cancer], colonic diverticulitis, colonic diverticulosis, megacolon [Hirschsprung disease, toxic megacolon]; sigmoid diseases [proctocolitis, sigmoin neoplasms]), constipation, Crohn's disease, diarrhea (infantile diarrhea, dysentery), duodenal diseases (duodenal neoplasms, duodenal obstruction, duodenal ulcer, duodenitis), enteritis (enterocolitis), HIV enteropathy, ileal diseases (ileal neoplasms, ileitis), immunoproliferative small intestinal disease, inflammatory bowel disease (ulcerative colitis, Crohn's disease), intestinal stenosis, parasitic diseases (anisakiasis, balantidiasis, blastocystis infections, cryptosporidiosis, dientamoebiasis, amebic dysentery, giardiasis), intestinal fistula (rectal fistula), intestinal neoplasms (cecal neoplasms, colonic neoplasms, duodenal neoplasms, ileal neoplasms, intestinal polyps, jejunal neoplasms, rectal neoplasms), intestinal obstruction (afferent loop syndrome, duodenal obstruction, impacted feces, intestinal pseudo-obstruction [cecal volvulus], intussusception), intestinal perforation, intestinal polyps (colonic polyps, gardner syndrome, peutz-jeghers syndrome), jejunal diseases (jejunal neoplasms), malabsorption syndromes (blind loop syndrome, celiac disease, lactose intolerance, short bowel syndrome, tropical sprue, whipple's disease), mesenteric vascular occlusion, pneumatosis cystoides intestinalis, protein-losing enteropathies (intestinal lymphangiectasis), rectal diseases (anus diseases, fecal incontinence, hemorrhoids, proctitis, rectal fistula, rectal prolapse,

rectocele), peptic ulcer (duodenal ulcer, peptic esophagitis, hemorrhage, perforation, stomach ulcer, Zollinger-Ellison syndrome), postgastrectomy syndromes (dumping syndrome), stomach diseases (e.g., achlorhydria, duodenogastric reflux (bile reflux), gastric antral vascular ectasia, gastric fistula, gastric outlet obstruction, gastritis (atrophic or hypertrophic), gastroparesis, stomach dilatation, stomach diverticulum, stomach neoplasms (gastric cancer, gastric polyps, gastric adenocarcinoma, hyperplastic gastric polyp), stomach rupture, stomach ulcer, stomach volvulus), tuberculosis, visceroptosis, vomiting (e.g., hematemesis, hyperemesis gravidarum, postoperative nausea and vomiting) and hemorrhagic colitis.

[0812] Further diseases and/or disorders of the gastrointestinal system include biliary tract diseases, such as, gastroschisis, fistula (e.g., biliary fistula, esophageal fistula, gastric fistula, intestinal fistula, pancreatic fistula), neoplasms (e.g., biliary tract neoplasms, esophageal neoplasms, such as adenocarcinoma of the esophagus, esophageal squamous cell carcinoma, gastrointestinal neoplasms, pancreatic neoplasms, such as adenocarcinoma of the pancreas, mucinous cystic neoplasm of the pancreas, pancreatic cystic neoplasms, pancreatoblastoma, and peritoneal neoplasms), esophageal disease (e.g., bullous diseases, candidiasis, glycogenic acanthosis, ulceration, barrett esophagus varices, stenosis, cyst, diverticulum (e.g., Zenker's diverticulum), fistula (e.g., tracheoesophageal fistula), motility disorders (e.g., CREST syndrome, deglutition disorders, achalasia, spasm, gastroesophageal reflux), neoplasms, perforation (e.g., Boerhaave syndrome, Mallory-Weiss syndrome), stenosis, esophagitis, diaphragmatic hernia (e.g., hiatal hernia); gastrointestinal diseases, such as, gastroenteritis (e.g., cholera morbus, norwalk virus infection), hemorrhage (e.g., hematemesis, melena, peptic ulcer hemorrhage), stomach neoplasms (gastric cancer, gastric polyps, gastric adenocarcinoma, stomach cancer)), hernia (e.g., congenital diaphragmatic hernia, femoral hernia, inguinal hernia, obturator hernia, umbilical hernia, ventral hernia), and intestinal diseases (e.g., cecal diseases (appendicitis, cecal neoplasms)).

Chemotaxis

[0813] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may have chemotaxis activity. A chemotaxic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

[0814] Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may increase chemotaxic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotaxic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat wounds.

[0815] It is also contemplated that fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention could be used as an inhibitor of chemotaxis.

Binding Activity

[0816] Albumin fusion proteins of the invention may be used to screen for molecules that bind to the Therapeutic protein portion of the fusion protein or for molecules to which the Therapeutic protein portion of the fusion protein binds. The binding of the fusion protein and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the fusion protein or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

[0817] Preferably, the molecule is closely related to the natural ligand of the Therapeutic protein portion of the fusion protein of the invention, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991)). Similarly, the molecule can be closely related to the natural receptor to which the Therapeutic protein portion of an albumin fusion protein of the invention binds, or at least, a fragment of the receptor capable of being bound by the Therapeutic protein portion of an albumin fusion protein of the invention (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

[0818] Preferably, the screening for these molecules involves producing appropriate cells which express the albumin fusion proteins of the invention. Preferred cells include cells from mammals, yeast, Drosophila, or *E. coli*.

[0819] The assay may simply test binding of a candidate compound to an albumin fusion protein of the invention, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the fusion protein.

[0820] Alternatively, the assay can be carried out using cell-free preparations, fusion protein/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing an albumin fusion protein, measuring fusion protein/molecule activity or binding, and comparing the fusion protein/molecule activity or binding to a standard.

[0821] Preferably, an ELISA assay can measure fusion protein level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure fusion protein level or activity by either binding, directly or indirectly, to the albumin fusion protein or by competing with the albumin fusion protein for a substrate.

[0822] Additionally, the receptor to which a Therapeutic protein portion of an albumin fusion protein of the invention binds can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). For example, in cases wherein the Therapeutic protein portion of the fusion protein corresponds to FGF, expression cloning may be employed wherein polyadenylated RNA is prepared from a cell responsive to the albumin fusion protein, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the albumin fusion protein. Transfected cells which are grown on glass slides are exposed to the albumin fusion protein of the present invention, after they have been labeled. The albumin fusion proteins can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

[0823] Following fixation and incubation, the slides are subjected to auto-radiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.

[0824] As an alternative approach for receptor identification, a labeled albumin fusion protein can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule for the Therapeutic protein component of an albumin fusion protein of the invention, the linked material may be resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the fusion protein can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

[0825] Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of the fusion protein, and/or Therapeutic protein portion or albumin component of an albumin fusion protein of the present invention, thereby effectively generating agonists and antagonists of an albumin fusion protein of the present invention. *See generally*, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P. A., *et al.*, *Curr. Opinion Biotechnol.* 8:724-33 (1997); Harayama, S. *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, L. O., *et al.*, *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. *Biotechniques* 24(2):308-13 (1998); each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides encoding albumin fusion proteins of the invention and thus, the albumin fusion proteins encoded thereby, may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired molecule by homologous, or site-specific, recombination. In another embodiment, polynucleotides encoding albumin fusion proteins of the invention and thus, the albumin fusion proteins encoded thereby, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of an albumin fusion protein of the present invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments, the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs), nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

[0826] Other preferred fragments are biologically active fragments of the Therapeutic protein portion and/or albumin component of the albumin fusion proteins of the present invention. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of a Therapeutic protein portion and/or albumin component of the albumin fusion proteins of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

[0827] Additionally, this invention provides a method of screening compounds to identify those which modulate the action of an albumin fusion protein of the present invention. An example of such an assay comprises combining a mammalian fibroblast cell, an albumin fusion protein of the present invention, and the compound to be screened and ^{3}[H] thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation by determining the uptake of ^{3}[H] thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of ^{3}[H] thymidine. Both agonist and antagonist compounds may be identified by this procedure.

[0828] In another method, a mammalian cell or membrane preparation expressing a receptor for the Therapeutic protien component of a fusion protine of the invention is incubated with a labeled fusion protein of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of a compound to be screened and the receptor is measured and the ability of the compound to bind to the receptor and elicit a second

messenger response is measured to determine if the compound is a potential fusion protein. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

[0829] All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the fusion protein/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the albumin fusion proteins of the invention from suitably manipulated cells or tissues.

[0830] Therefore, the invention includes a method of identifying compounds which bind to an albumin fusion protein of the invention comprising the steps of: (a) incubating a candidate binding compound with an albumin fusion protein of the present invention; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with an albumin fusion protein of the present invention, (b) assaying a biological activity, and (b) determining if a biological activity of the fusion protein has been altered.

Targeted Delivery

[0831] In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a receptor for a component of an albumin fusion protein of the invention.

[0832] As discussed herein, fusion proteins of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering fusion proteins of the invention (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a Therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

[0833] In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering an albumin fusion protein of the invention (e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

[0834] By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNAse, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubicin, and phenoxyacetamide derivatives of doxorubicin.

Drug Screening

[0835] Further contemplated is the use of the albumin fusion proteins of the present invention, or the polynucleotides encoding these fusion proteins, to screen for molecules which modify the activities of the albumin fusion protein of the present invention or proteins corresponding to the Therapeutic protein portion of the albumin fusion protein. Such a method would include contacting the fusion protein with a selected compound(s) suspected of having antagonist or agonist activity, and assaying the activity of the fusion protein following binding.

[0836] This invention is particularly useful for screening therapeutic compounds by using the albumin fusion proteins of the present invention, or binding fragments thereof, in any of a variety of drug screening techniques. The albumin fusion protein employed in such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the albumin fusion protein. Drugs are screened against such transformed cells or supernatants obtained from culturing such cells, in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and an albumin fusion protein of the present invention.

[0837] Thus, the present invention provides methods of screening for drugs or any other agents which affect activities mediated by the albumin fusion proteins of the present invention. These methods comprise contacting such an agent with an albumin fusion protein of the present invention or a fragment thereof and assaying for the presence of a complex between the agent and the albumin fusion protein or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a particular agent to bind to the albumin fusion protein of the present invention.

[0838] Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to an albumin

fusion protein of the present invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with an albumin fusion protein of the present invention and washed. Bound peptides are then detected by methods well known in the art. Purified albumin fusion protein may be coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

[0839] This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding an albumin fusion protein of the present invention specifically compete with a test compound for binding to the albumin fusion protein or fragments thereof. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic epitopes with an albumin fusion protein of the invention.

Binding Peptides and Other Molecules

[0840] The invention also encompasses screening methods for identifying polypeptides and nonpolypeptides that bind albumin fusion proteins of the invention, and the binding molecules identified thereby. These binding molecules are useful, for example, as agonists and antagonists of the albumin fusion proteins of the invention. Such agonists and antagonists can be used, in accordance with the invention, in the therapeutic embodiments described in detail, below.

[0841] This method comprises the steps of: contacting an albumin fusion protein of the invention with a plurality of molecules; and identifying a molecule that binds the albumin fusion protein.

[0842] The step of contacting the albumin fusion protein of the invention with the plurality of molecules may be effected in a number of ways. For example, one may contemplate immobilizing the albumin fusion protein on a solid support and bringing a solution of the plurality of molecules in contact with the immobilized polypeptides. Such a procedure would be akin to an affinity chromatographic process, with the affinity matrix being comprised of the immobilized albumin fusion protein of the invention. The molecules having a selective affinity for the albumin fusion protein can then be purified by affinity selection. The nature of the solid support, process for attachment of the albumin fusion protein to the solid support, solvent, and conditions of the affinity isolation or selection are largely conventional and well known to those of ordinary skill in the art.

[0843] Alternatively, one may also separate a plurality of polypeptides into substantially separate fractions comprising a subset of or individual polypeptides. For instance, one can separate the plurality of polypeptides by gel electrophoresis, column chromatography, or like method known to those of ordinary skill for the separation of polypeptides. The individual polypeptides can also be produced by a transformed host cell in such a way as to be expressed on or about its outer surface (e.g., a recombinant phage). Individual isolates can then be "probed" by an albumin fusion protein of the invention, optionally in the presence of an inducer should one be required for expression, to determine if any selective affinity interaction takes place between the albumin fusion protein and the individual clone. Prior to contacting the albumin fusion protein with each fraction comprising individual polypeptides, the polypeptides could first be transferred to a solid support for additional convenience. Such a solid support may simply be a piece of filter membrane, such as one made of nitrocellulose or nylon. In this manner, positive clones could be identified from a collection of transformed host cells of an expression library, which harbor a DNA construct encoding a polypeptide having a selective affinity for an albumin fusion protein of the invention. Furthermore, the amino acid sequence of the polypeptide having a selective affinity for an albumin fusion protein of the invention can be determined directly by conventional means or the coding sequence of the DNA encoding the polypeptide can frequently be determined more conveniently. The primary sequence can then be deduced from the corresponding DNA sequence. If the amino acid sequence is to be determined from the polypeptide itself, one may use microsequencing techniques. The sequencing technique may include mass spectroscopy.

[0844] In certain situations, it may be desirable to wash away any unbound polypeptides from a mixture of an albumin fusion protein of the invention and the plurality of polypeptides prior to attempting to determine or to detect the presence of a selective affinity interaction. Such a wash step may be particularly desirable when the albumin fusion protein of the invention or the plurality of polypeptides are bound to a solid support.

[0845] The plurality of molecules provided according to this method may be provided by way of diversity libraries, such as random or combinatorial peptide or nonpeptide libraries which can be screened for molecules that specifically bind an albumin fusion protein of the invention. Many libraries are known in the art that can be used, e.g., chemically synthesized libraries, recombinant (e.g., phage display libraries), and *in vitro* translation-based libraries. Examples of chemically synthesized libraries are described in Fodor et al., Science 251:767-773 (1991); Houghten et al., Nature 354:84-86 (1991); Lam et al., Nature 354:82-84 (1991); Medynski, Bio/Technology 12:709-710 (1994); Gallop et al., J. Medicinal Chemistry 37(9):1233-1251 (1994); Ohlmeyer et al., Proc. Natl. Acad. Sci. USA 90:10922-10926 (1993); Erb et al., Proc. Natl. Acad. Sci. USA 91:11422-11426 (1994); Houghten et al., Biotechniques 13:412 (1992); Jayawickreme et al., Proc. Natl. Acad. Sci. USA 91:1614-1618 (1994); Salmon et al., Proc. Natl. Acad. Sci. USA 90:11708-11712 (1993); PCT Publication No. WO 93/20242; and Brenner and Lerner, Proc. Natl. Acad. Sci. USA 89:5381-5383 (1992).

[0846] Examples of phage display libraries are described in Scott et al., Science 249:386-390 (1990); Devlin et al., Science, 249:404-406 (1990); Christian et al., 1992, J. Mol. Biol. 227:711-718 1992); Lenstra, J. Immunol. Meth. 152:149-157 (1992); Kay et al., Gene 128:59-65 (1993); and PCT Publication No. WO 94/18318 dated Aug. 18, 1994.

[0847] In vitro translation-based libraries include but are not limited to those described in PCT Publication No. WO 91/05058 dated Apr. 18, 1991; and Matthakis et al., Proc. Natl. Acad. Sci. USA 91:9022-9026 (1994).

[0848] By way of examples of nonpeptide libraries, a benzodiazepine library (see e.g., Bunin et al., Proc. Natl. Acad. Sci. USA 91:4708-4712 (1994)) can be adapted for use. Peptoid libraries (Simon et al., Proc. Natl. Acad. Sci. USA 89:9367-9371 (1992)) can also be used. Another example of a library that can be used, in which the amide functionalities in peptides have been permethylated to generate a chemically transformed combinatorial library, is described by Ostresh et al. (Proc. Natl. Acad. Sci. USA 91:11138-11142 (1994)).

[0849] The variety of non-peptide libraries that are useful in the present invention is great. For example, Ecker and Crooke (Bio/Technology 13:351-360 (1995) list benzodiazepines, hydantoins, piperazinediones, biphenyls, sugar analogs, beta-mercaptoketones, arylacetic acids, acylpiperidines, benzopyrans, cubanes, xanthines, aminimides, and oxazolones as among the chemical species that form the basis of various libraries.

[0850] Non-peptide libraries can be classified broadly into two types: decorated monomers and oligomers. Decorated monomer libraries employ a relatively simple scaffold structure upon which a variety functional groups is added. Often the scaffold will be a molecule with a known useful pharmacological activity. For example, the scaffold might be the benzodiazepine structure.

[0851] Non-peptide oligomer libraries utilize a large number of monomers that are assembled together in ways that create new shapes that depend on the order of the monomers. Among the monomer units that have been used are carbamates, pyrrolinones, and morpholinos. Peptoids, peptide-like oligomers in which the side chain is attached to the alpha amino group rather than the alpha carbon, form the basis of another version of non-peptide oligomer libraries. The first non-peptide oligomer libraries utilized a single type of monomer and thus contained a repeating backbone. Recent libraries have utilized more than one monomer, giving the libraries added flexibility.

[0852] Screening the libraries can be accomplished by any of a variety of commonly known methods. See, e.g., the following references, which disclose screening of peptide libraries: Parmley et al., Adv. Exp. Med. Biol. 251:215-218 (1989); Scott et al., Science 249:386-390 (1990); Fowlkes et al., BioTechniques 13:422-427 (1992); Oldenburg et al., Proc. Natl. Acad. Sci. USA 89:5393-5397 (1992); Yu et al., Cell 76:933-945 (1994); Staudt et al., Science 241:577-580 (1988); Bock et al., Nature 355:564-566 (1992); Tuerk et al., Proc. Natl. Acad. Sci. USA 89:6988-6992 (1992); Ellington et al., Nature 355:850-852 (1992); U.S. Pat. No. 5,096,815, U.S. Pat. No. 5,223,409, and U.S. Pat. No. 5,198,346, all to Ladner et al.; Rebar et al., Science 263:671-673 (1993); and PCT Publication No. WO 94/18318.

[0853] In a specific embodiment, screening to identify a molecule that binds an albumin fusion protein of the invention can be carried out by contacting the library members with an albumin fusion protein of the invention immobilized on a solid phase and harvesting those library members that bind to the albumin fusion protein. Examples of such screening methods, termed "panning" techniques are described by way of example in Parmley et al., Gene 73:305-318 (1988); Fowlkes et al., BioTechniques 13:422-427 (1992); PCT Publication No. WO 94/18318; and in references cited herein.

[0854] In another embodiment, the two-hybrid system for selecting interacting proteins in yeast (Fields et al., Nature 340:245-246 (1989); Chien et al., Proc. Natl. Acad. Sci. USA 88:9578-9582 (1991) can be used to identify molecules that specifically bind to polypeptides of the invention.

[0855] Where the binding molecule is a polypeptide, the polypeptide can be conveniently selected from any peptide library, including random peptide libraries, combinatorial peptide libraries, or biased peptide libraries. The term "biased" is used herein to mean that the method of generating the library is manipulated so as to restrict one or more parameters that govern the diversity of the resulting collection of molecules, in this case peptides.

[0856] Thus, a truly random peptide library would generate a collection of peptides in which the probability of finding a particular amino acid at a given position of the peptide is the same for all 20 amino acids. A bias can be introduced into the library, however, by specifying, for example, that a lysine occur every fifth amino acid or that positions 4, 8, and 9 of a decapeptide library be fixed to include only arginine. Clearly, many types of biases can be contemplated, and the present invention is not restricted to any particular bias. Furthermore, the present invention contemplates specific types of peptide libraries, such as phage displayed peptide libraries and those that utilize a DNA construct comprising a lambda phage vector with a DNA insert.

[0857] As mentioned above, in the case of a binding molecule that is a polypeptide, the polypeptide may have about 6 to less than about 60 amino acid residues, preferably about 6 to about 10 amino acid residues, and most preferably, about 6 to about 22 amino acids. In another embodiment, a binding polypeptide has in the range of 15-100 amino acids, or 20-50 amino acids.

[0858] The selected binding polypeptide can be obtained by chemical synthesis or recombinant expression.

Other Activities

[0859] An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention, may be employed in treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as thrombosis, arteriosclerosis, and other cardiovascular conditions. The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

[0860] An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and ulcers since they are mitogenic to various cells of different origins, such as fibroblast cells and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

[0861] An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may also be employed stimulate neuronal growth and to treat and prevent neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's disease, Parkinson's disease, and AIDS-related complex. An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may have the ability to stimulate chondrocyte growth, therefore, they may be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone grafts.

[0862] An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may be also be employed to prevent skin aging due to sunburn by stimulating keratinocyte growth.

[0863] An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may also be employed for preventing hair loss. Along the same lines, an albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may be employed to stimulate growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

[0864] An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues. An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

[0865] An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

[0866] An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, an albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

[0867] An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may be used to change a mammal's mental state or physical state by influencing biorhythms, caricadic rhythms, depression (including depressive disorders), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

[0868] An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

[0869] The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit, goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

[0870] Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

[0871] Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the alterations detected in the present invention and practice the claimed methods. The following working examples therefore, specifically point out preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

EXAMPLES

EXAMPLE 1: Generation of pScNHSA and pScCHSA.

[0872] The vectors pScNHSA (ATCC Deposit No. PTA-3279) and pScCHSA (ATCC Deposit No. PTA-3276) are derivatives of pPPC0005 (ATCC Deposit No. PTA-3278) and are used as cloning vectors into which polynucleotides encoding a therapeutic protein or fragment or variant thereof is inserted adjacent to and in translation frame with polynucleotides encoding human serum albumin "HSA". pScCHSA may be used for generating Therapeutic protein-HSA fusions, while pScNHSA may be used to generate HSA-Therapeutic protein fusions.

Generation of pScCHSA: albumin fusion with the albumin moiety C-terminal to the therapeutic portion.

[0873] A vector to facilitate cloning DNA encoding a Therapeutic protein N-terminal to DNA encoding the mature albumin protein was made by altering the nucleic acid sequence that encodes the chimeric HSA signal peptide in pPPC0005 to include the *Xho* I and *Cla* I restriction sites.

[0874] First, the *Xho* I and *Cla* I sites inherent to pPPC0005 (located 3' of the ADH1 terminator sequence) were eliminated by digesting pPPC0005 with *Xho* I and *Cla* I, filling in the sticky ends with T4 DNA polymerase, and religating the blunt ends to create pPPC0006.

[0875] Second, the *Xho* I and *Cla* I restriction sites were engineered into the nucleic acid sequence that encodes the signal peptide of HSA (a chimera of the HSA leader and a kex2 site from mating factor alpha, "MAF") in pPPC0006 using two rounds of PCR. In the first round of PCR, amplification with primers shown as SEQ ID NO:36 and SEQ ID NO:37 was performed. The primer whose sequence is shown as SEQ ID NO:36 comprises a nucleic acid sequence that encodes part of the signal peptide sequence of HSA, a kex2 site from the mating factor alpha leader sequence, and part of the amino-terminus of the mature form of HSA. Four point mutations were introduced in the sequence, creating the *Xho* I and *Cla* I sites found at the junction of the chimeric signal peptide and the mature form of HSA. These four mutations are underlined in the sequence shown below. In pPPC0005 the nucleotides at these four positions from 5' to 3' are T, G, T, and G.

5'-GCCTCGAGAAAAGAGATGCACACAAGAGTGAGGTTGCTCATCGATTTAAAGATTTGGG-3' (SEQ ID NO:36) and

5'-AATCGATGAGCAACCTCACTCTTGTGTGCATCTCTTTTCTCGAGGCTCCTGGAATAAGC-3' (SEQ ID NO:37). A second round of PCR was then performed with an upstream flanking primer, 5'-TACAAACTTAAGAGTCCAATTAGC-3' (SEQ ID NO:38) and a downstream flanking primer 5'-CACTTCTCTAGAGTGGTTTCATATGTCTT-3' (SEQ ID NO:39). The resulting PCR product was then purified and digested with *Afl* II and *Xba* I and ligated into the same sites in pPPC0006 creating pScCHSA. The resulting plasmid has *Xho* I and *Cla* I sites engineered into the signal sequence. The presence of the *Xho* I site creates a single amino acid change in the end of the signal sequence from LDKR to LEKR. The D to E change will not be present in the final albumin fusion protein expression plasmid when a nucleic acid sequence comprising a polynucleotide encoding the Therapeutic portion of the albumin fusion protein with a 5' *Sal* I site (which is compatible with the *Xho* I site) and a 3' *Cla* I site is ligated into the *Xho* I and *Cla* I sites of pScCHSA. Ligation of *Sal* I to *Xho* I restores the original amino acid sequence of the signal peptide sequence. DNA encoding the Therapeutic portion of the albumin fusion protein may be inserted after the Kex2 site (Kex2 cleaves after the dibasic amino acid sequence KR at the end of the signal peptide) and prior to the *Cla* I site.

Generation of pScNHSA: albumin fusion with the albumin moiety N-terminal to the therapeutic portion.

[0876] A vector to facilitate cloning DNA encoding a Therapeutic protein portion C-terminal to DNA encoding the mature albumin protein, was made by adding three, eight-base-pair restriction sites to pScCHSA. The *Asc* I, *Fse* I, and *Pme* I restriction sites were added in between the *Bsu36* I and *Hind* III sites at the end of the nucleic acid sequence encoding the mature HSA protein. This was accomplished through the use of two complementary synthetic primers containing the *Asc* I, *Fse* I, and *Pme* I restriction sites underlined (SEQ ID NO:40 and SEQ ID NO:41). 5'-AAGCTGCCTTAGGCTTATAATAAGGCGCGCCGGCCGGCCGTTTAAACTAAGCTTAATTCT-3' (SEQ ID NO:40) and

5-AGAATTAAGCTTAGTTTAAACGGCCGGCCGGCGCGCCTTATTATAAGCCTAAGGCAGCTT-3' (SEQ ID NO:41). These primers were annealed and digested with *Bsu36* I and *Hind* III and ligated into the same sites in pScCHSA creating pScNHSA.

EXAMPLE 2: General Construct Generation for Yeast Transformation.

[0877] The vectors pScNHSA and pScCHSA may be used as cloning vectors into which polynucleotides encoding a therapeutic protein or fragment or variant thereof is inserted adjacent to polynucleotides encoding mature human serum albumin "HSA". pScCHSA is used for generating Therapeutic protein-HSA fusions, while pScNHSA may be used to generate HSA-Therapeutic protein fusions.

Generation of albumin fusion constructs comprising HSA-Therapeutic protein fusion products.

[0878] DNA encoding a Therapeutic protein (e.g., sequences shown in SEQ ID NO:X or known in the art) may be PCR amplified using the primers which facilitate the generation of a fusion construct (e.g., by adding restriction sites, encoding seamless fusions, encoding linker sequences, etc.) For example, one skilled in the art could design a 5' primer that adds polynucleotides encoding the last four amino acids of the mature form of HSA (and containing the *Bsu36*I site) onto the 5' end of DNA encoding a Therapeutic protein; and a 3' primer that adds a STOP codon and appropriate cloning sites onto the 3' end of the Therapeutic protein coding sequence. For instance, the forward primer used to amplify DNA encoding a Therapeutic protein might have the sequence, 5'-aagctGCCTTAGGCTTA$(N)_{15}$-3' (SEQ ID NO:42) where the underlined sequence is a *Bsu36*I site, the upper case nucleotides encode the last four amino acids of the mature HSA protein (ALGL), and $(N)_{15}$ is identical to the first 15 nucleotides encoding the Therapeutic protein of interest. Similarly, the reverse primer used to amplify DNA encoding a Therapeutic protein might have the sequence, 5'-GCGCGC*GTTTAAAC*GGCCGGCCGGCGCGCCTTATTA$(N)_{15}$-3' (SEQ ID NO:43) where the italicized sequence is a *Pme* I site, the double underlined sequence is an *Fse* I site, the singly underlined sequence is an *Asc* I site, the boxed nucleotides are the reverse complement of two tandem stop codons, and $(N)_{15}$ is identical to the reverse complement of the last 15 nucleotides encoding the Therapeutic protein of interest. Once the PCR product is amplified it may be cut with *Bsu36*I and one of (*Asc* I, *Fse* I, or *Pme* I) and ligated into pScNHSA.

[0879] The presence of the *Xho* I site in the HSA chimeric leader sequence creates a single amino acid change in the end of the chimeric signal sequence, i.e. the HSA-kex2 signal sequence, from LDKR (SEQ ID NO:44) to LEKR (SEQ ID NO:45).

Generation of albumin fusion constructs comprising gene-HSA fusion products.

[0880] Similar to the method described above, DNA encoding a Therapeutic protein may be PCR amplified using the following primers: A 5' primer that adds polynucleotides containing a *Sal*I site and encoding the last three amino acids of the HSA leader sequence, DKR, onto the 5' end of DNA encoding a Therapeutic protein; and a 3' primer that adds polynucleotides encoding the first few amino acids of the mature HSA containing a

Cla I site onto the 3' end of DNA encoding a Therapeutic protein. For instance, the forward primer used to amplify the DNA encoding a Therapeutic protein might have the sequence, 5'-aggagatcGACAAAAGA$(N)_{15}$-3' (SEQ ID NO:46) where the underlined sequence is a *Sal* I site, the upper case nucleotides encode the last three amino acids of the HSA leader sequence (DKR), and $(N)_{15}$ is identical to the first 15 nucleotides encoding the Therapeutic protein of interest. Similarly, the reverse primer used to amplify the DNA encoding a Therapeutic protein might have the sequence, 5'-CTTTAA*ATCGAT*GAGCAAGCTCACTCTTGTGTGCATC$(N)_{15}$-3'(SEQ ID NO:47) where the italicized sequence is a *Cla* I site, the underlined nucleotides are the reverse complement of the DNA encoding the first 9 amino acids of the mature form of HSA (DAHKSEVAH, SEQ ID NO:48), and $(N)_{15}$ is identical to the reverse complement of the last 15 nucleotides encoding the Therapeutic protein of interest. Once the PCR product is amplified it may be cut with *Sal* I and *Cla* I and ligated into pScCHSA digested with *Xho* I and *Cla* I. A different signal or leader sequence may be desired, for example, invertase "INV" (Swiss-Prot Accession P00724), mating factor alpha "MAF" (Genbank Accession AAA18405), MPIF (Genseq AAF82936), Fibulin B (Swiss-Prot Accession P23142), Clusterin (Swiss-Prot Accession P10909), Insulin-Like Growth Factor- Binding Protein 4 (Swiss-Prot Accession P22692), and permutations of the HSA leader sequence can be subcloned into the appropriate vector by means of standard methods known in the art.

Generation of albumin fusion construct compatible for expression in yeast S. cerevisiae.

[0881] The *Not* I fragment containing the DNA encoding either an N-terminal or C-terminal albumin fusion protein generated from pScNHSA or pScCHSA may then be cloned into the *Not* I site of pSAC35 which has a LEU2 selectable marker. The resulting vector is then used in transformation of a yeast *S. cerevisiae* expression system.

EXAMPLE 3: General Expression in Yeast S. cerevisiae.

[0882] An expression vector compatible with yeast expression can be transformed into yeast *S. cerevisiae* by lithium acetate transformation, electroporation, or other methods known in the art and or as described in part in Sambrook, Fritsch, and Maniatis. 1989. "Molecular Cloning: A Laboratory Manual, 2nd edition", volumes 1-3, and in Ausubel et al. 2000. Massachusetts General Hospital and Harvard Medical School "Current Protocols in Molecular Biology", volumes 1-4. The expression vectors are introduced into *S. cerevisiae* strains DXY1, D88, or BXP10 by transformation, individual transformants can be grown, for example, for 3 days at 30°C in 10 mL YEPD (1% w/v yeast extract, 2 % w/v, peptone, 2 % w/v, dextrose), and cells can be collected at stationary phase after 60 hours of growth. Supernatants are collected by clarifying cells at 3000g for 10 minutes.

[0883] pSAC35 (Sleep et al., 1990, Biotechnology 8:42 and see Figure 3) comprises, in addition to the LEU2 selectable marker, the entire yeast 2 μm plasmid to provide replication functions, the PRB1 promoter, and the ADH1 termination signal.

EXAMPLE 4: General Purification of an Albumin Fusion Protein Expressed from an Albumin Fusion in Yeast S. cerevisiae.

[0884] In preferred embodiments, albumin fusion proteins of the invention comprise the mature form of HSA fused to either the N- or C-terminus of the mature form of a therapeutic protein or portions thereof (e.g., the mature form of a therapeutic protein listed in Table 1, or the mature form of a therapeutic protein shown in Table 2 as SEQ ID NO:2). In one embodiment of the invention, albumin fusion proteins of the invention further comprise a signal sequence which directs the nascent fusion polypeptide in the secretory pathways of the host used for expression. In a preferred embodiment, the signal peptide encoded by the signal sequence is removed, and the mature albumin fusion protein is secreted directly into the culture medium. Albumin fusion proteins of the invention preferably comprise heterologous signal sequences (e.g., the non-native signal sequence of a particular therapeutic protein) including, but not limited to, MAF, INV, Ig, Fibulin B, Clusterin, Insulin-Like Growth Factor Binding Protein 4, variant HSA leader sequences including, but not limited to, a chimeric HSA/MAF leader sequence, or other heterologous signal sequences known in the art. Especially preferred are those signal sequence listed in Table 2 and/or the signal sequence listed in the "Expression of Fusion Proteins" and/or "Additional Methods of Recombinant and Synthetic Production of Albumin Fusion Proteins" section of the specification, above. In preferred embodiments, the fusion proteins of the invention further comprise an N-terminal methionine residue. Polynucleotides encoding these polypeptides, including fragments and/or variants, are also encompassed by the invention.

[0885] Albumin fusion proteins expressed in yeast as described above can be purified on a small-scale over a Dyax peptide affinity column as follows. Supernatants from yeast expressing an albumin fusion protein is diafiltrated against 3 mM phosphate buffer pH 6.2, 20 mM NaCl and 0.01% Tween 20 to reduce the volume and to remove the pigments. The solution is then filtered through a 0.22 μm device. The filtrate is loaded onto a Dyax peptide affinity column. The column is eluted with 100 mM Tris/HCl, pH 8.2 buffer. The peak fractions containing protein are collected and analyzed on SDS-PAGE after concentrating 5-fold.

[0886] For large scale purification, the following method can be utilized. The supernatant in excess of 2 L is diafiltered and concentrated to 500 mL in 20 mM Tris/HCl pH 8.0. The concentrated protein solution is loaded onto a pre-equilibrated 50 mL DEAE-Sepharose Fast Flow column, the column is washed, and the protein is eluted with a linear gradient of NaCl from 0 to 0.4 M NaCl in 20 mM Tris/HCl, pH 8.0. Those fractions containing the protein are pooled, adjusted to pH 6.8 with 0.5 M sodium phosphate (NaH_2PO_4). A final concentration of 0.9 M $(NH_4)_2SO_4$ is added to the protein solution and the whole solution is loaded onto a pre-equilibrated 50 mL Butyl650S column. The protein is eluted with a linear gradient of ammonium sulfate (0.9 to 0 M $(NH_4)_2SO_4$). Those fractions with the albumin fusion are again pooled, diafiltered against 10 mM Na_2HPO_4/citric acid buffer pH 5.75, and loaded onto a 50 mL pre-equilibrated SP-Sepharose Fast Flow column. The protein is eluted with a NaCl

linear gradient from 0 to 0.5 M. The fractions containing the protein of interest are combined, the buffer is changed to 10 mM Na_2HPO_4/citric acid pH 6.25 with an Amicon concentrator, the conductivity is < 2.5 mS/cm. This protein solution is loaded onto a 15 mL pre-equilibrated Q-Sepharose high performance column, the column is washed, and the protein is eluted with a NaCl linear gradient from 0 to 0.15 M NaCl. The purified protein can then be formulated into a specific buffer composition by buffer exchange.

EXAMPLE 5: General Construct Generation for Mammalian Cell Transfection.

Generation of albumin fusion construct compatible for expression in mammalian cell-lines.

[0887] Albumin fusion constructs can be generated in expression vectors for use in mammalian cell culture systems. DNA encoding a therapeutic protein can be cloned N-terminus or C-terminus to HSA in a mammalian expression vector by standard methods known in the art (e.g., PCR amplification, restriction digestion, and ligation). Once the expression vector has been constructed, transfection into a mammalian expression system can proceed. Suitable vectors are known in the art including, but not limited to, for example, the pC4 vector, and/or vectors available from Lonza Biologics, Inc. (Portsmouth, NH).

[0888] The DNA encoding human serum albumin has been cloned into the pC4 vector which is suitable for mammalian culture systems, creating plasmid pC4:HSA (ATCC Deposit # PTA-3277). This vector has a DiHydroFolate Reductase, "DHFR", gene that will allow for selection in the presence of methotrexate.

[0889] The pC4:HSA vector is suitable for expression of albumin fusion proteins in CHO cells. For expression, in other mammalian cell culture systems, it may be desirable to subclone a fragment comprising, or alternatively consisting of, DNA which encodes for an albumin fusion protein into an alternative expression vector. For example, a fragment comprising, or alternatively consisting, of DNA which encodes for a mature albumin fusion protein may be subcloned into another expression vector including, but not limited to, any of the mammalian expression vectors described herein.

[0890] In a preferred embodiment, DNA encoding an albumin fusion construct is subcloned into vectors provided by Lonza Biologics, Inc. (Portsmouth, NH) by procedures known in the art for expression in NS0 cells.

Generation of albumin fusion constructs comprising HSA-Therapeutic Protein fusion products.

[0891] Using pC4:HSA (ATCC Deposit # PTA-3277), albumin fusion constructs can be generated in which the Therapeutic protein portion is C terminal to the mature albumin sequence. For example, one can clone DNA encoding a Therapeutic protein of fragment or variant thereof between the *Bam* 36I and *Asc* I restriction sites of the vector. When cloning into the *Bam* 36I and *Asc* I, the same primer design used to clone into the yeast vector system (SEQ ID NO:42 and 43) may be employed (see Example 2).

Generation of albumin fusion constructs comprising some-HSA fusion products.

[0892] Using pC4:HSA (ATCC Deposit # PTA-3277), albumin fusion constructs can be generated in which a Therapeutic protein portion is cloned N terminal to the mature albumin sequence. For example, one can clone DNA encoding a Therapeutic protein that has its own signal sequence between the *Bam* HI (or *Hind* III) and *Cla* I sites of pC4:HSA. When cloning into either the *Bam* HI or *Hind* III site, it is preferrable to include a Kozak sequence (CCGCCACCATG, SEQ ID NO:49) prior to the translational start codon of the DNA encoding the Therapeutic protein. If a Therapeutic protein does not have a signal sequence, DNA encoding that Therapeutic protein may be cloned in between the *Xho* I and *Cla* I sites of pC4:HSA. When using the *Xho* I site, the following 5' (SEQ ID NO:50) and 3' (SEQ ID NO:51) exemplary PCR primers may be used:

5'-CCGCCGCTCGAGGGGTGTGTTTCGTCGA$(N)_{10}$-3' (SEQ ID NO: 50)

5'-AGTCCCATCGATGAGCAACCTCACTCTTGTGTGCATC$(N)_{10}$-3' (SEQ ID NO:51)

[0893] In the 5' primer (SEQ ID NO:50), the underlined sequence is a *Xho* I site; and the *Xho* I site and the DNA following the *Xho* I site code for the last seven amino acids of the leader sequence of natural human serum albumin. In SEQ ID NO:50, "$(N)_{10}$" is DNA identical to the first 10 nucleotides encoding the Therapeutic protein of interest. In the 3' primer (SEQ ID NO:51), the underlined sequence is a *Cla* I site; and the *Cla* I site and the DNA following it are the reverse complement of the DNA encoding the first 10 amino acids of the mature HSA protein (SEQ ID NO:1). In SEQ ID NO:51 "$(N)_{10}$" is the reverse complement of DNA encoding the last 10 nucleotides encoding the Therapeutic protein of interest. Using these two primers, one may PCR amplify the Therapeutic protein of interest, purify the PCR product, digest it with *Xho* I and *Cla* I restriction enzymes and clone it into the *Xho* I and *Cla* I sites in the pC4:HSA vector.

[0894] If an alternative leader sequence is desired, the native albumin leader sequence can be replaced with the chimeric albumin leader, i.e., the HSA-knx2 signal peptide, or an alternative leader by standard methods known in the art. (For example, one skilled in the art could routinely PCR amplify an alternate leader and subclone the PCR product into an albumin fusion construct in place of the albumin leader while maintaining the reading frame).

EXAMPLE 6: General Expression in Mammalian Cell-Lines.

[0895] An albumin fusion construct generated in an expression vector compatible with expression in mammalian cell-lines can be transfected into appropriate cell-lines by calcium phosphate precipitation, lipofectamine, electroporation, or other transfection methods known in the art and/or as described in Sambrook, Fritsch, and Maniatis. 1989. "Molecular Cloning: A Laboratory Manual, 2nd edition" and in Ausubel et al. 2000. Massachusetts General Hospital and Harvard Medical School "Current Protocols in Molecular Biology", volumes 1-4. The transfected cells are then

selected for by the presence of a selecting agent determined by the selectable marker in the expression vector.

[0896] The pC4 expression vector (ATCC Accession No. 209646) is a derivative of the plasmid pSV2-DHFR (ATCC Accession No. 37146). pC4 contains the strong promoter Long Terminal Repeats "LTR" of the Rous Sarcoma Virus (Cullen et al., March 1985, Molecular and Cellular Biology, 438-447) and a fragment of the CytoMegaloVirus "CMV"-enhancer (Boshart et al., 1985, Cell 41: 521-530). The vector also contains the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary "CHO" cells or other cell-lines lacking an active DHFR gene are used for transfection. Transfection of an albumin fusion construct in pC4 into CHO cells by methods known in the art will allow for the expression of the albumin fusion protein in CHO cells, followed by leader sequence cleavage, and secretion into the supernatant. The albumin fusion protein is then further purified from the supernatant.

[0897] The pEE12.1 expression vector is provided by Lonza Biologics, Inc. (Portsmouth, NH) and is a derivative of pEE6 (Stephens and Cockett, 1989, Nucl. Acids Res. 17: 7110). This vector comprises a promoter, enhancer and complete 5'-untranslated region of the Major Immediate Early gene of the human CytoMegaloVirus, "hCMV-MIE" (International Publication # WO89/01036), upstream of a sequence of interest, and a Glutamine Synthetase gene (Murphy et al., 1991, Biochem J. 227: 277-279; Bebbington et al., 1992, Bio/Technology 10:169-175; US patent US 5,122,464) for purposes of selection of transfected cells in selective methionine sulphoximine containing medium. Transfection of albumin fusion constructs made in pEE12.1 into NS0 cells (International Publication # WO86/05807) by methods known in the art will allow for the expression of the albumin fusion protein in NS0 cells, followed by leader sequence cleavage, and secretion into the supernatant. The albumin fusion protein is then further purified from the supernatant using techniques described herein or otherwise known in the art.

[0898] Expression of an albumin fusion protein may be analyzed, for example, by SDS-PAGE and Western blot, reversed phase HPLC analysis, or other methods known in the art.

[0899] Stable CHO and NS0 cell-lines transfected with albumin fusion constructs are generated by methods known in the art (e.g., lipofectamine transfection) and selected, for example, with 100 nM methotrexate for vectors having the DiHydroFolate Reductase 'DHFR' gene as a selectable marker or through growth in the absence of glutamine. Expression levels can be examined for example, by immunoblotting, primarily, with an anti-HSA serum as the primary antibody, or, secondarily, with serum containing antibodies directed to the Therapeutic protein portion of a given albumin fusion protein as the primary antibody.

[0900] Expression levels are examined by immunoblot detection with anti-HSA serum as the primary antibody. The specific productivity rates are determined via ELISA in which the capture antibody can be a monoclonal antibody towards the therapeutic protein portion of the albumin fusion and the detecting antibody can be the monoclonal anti-HSA-biotinylated antibody *(or vice versa)*, followed by horseradish peroxidase/streptavidin binding and analysis according to the manufacturer's protocol.

EXAMPLE 7: Expression of an Albumin Fusion Protein in Mammalian Cells.

[0901] The albumin fusion proteins of the present invention can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

[0902] Suitable expression vectors for use in practicing the present invention include, for example, vectors such as, pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSport 2.0, and pCMVSport 3.0. Mammalian host cells that could be used include, but are not limited to, human Hela, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

[0903] Alternatively, the albumin fusion protein can be expressed in stable cell lines containing the polynucleotide encoding the albumin fusion protein integrated into a chromosome. The co-transfection with a selectable marker such as DHFR, gpt, neomycin, or hygromycin allows the identification and isolation of the transfected cells.

[0904] The transfected polynucleotide encoding the fusion protein can also be amplified to express large amounts of the encoded fusion protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin et al., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page et al., Biotechnology 9:64-68 (1991)). Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., Biochem J. 227:277-279 (1991); Bebbington et al., Bio/Technology 10:169-175 (1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

[0905] Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No.209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology,

438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., Cell 41:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

[0906] Specifically, the plasmid pC6, for example, is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

[0907] A polynucleotide encoding an albumin fusion protein of the present invention is generated using techniques known in the art and this polynucleotide is amplified using PCR technology known in the art. If a naturally occurring signal sequence is used to produce the fusion protein of the present invention, the vector does not need a second signal peptide. Alternatively, if a naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., International Publication No. WO 96/34891.)

[0908] The amplified fragment encoding the fusion protein of the invention is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

[0909] The amplified fragment encoding the albumin fusion protein of the invention is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

[0910] Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five μg of the expression plasmid pC6 or pC4 is cotransfected with 0.5 μg of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 μM, 2 μM, 5 μM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 μM. Expression of the desired fusion protein is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

EXAMPLE 8: General Purification of an Albumin Fusion Protein Expressed from an Albumin Fusion Construct in Mammalian Cell-lines.

[0911] In preferred embodiments, albumin fusion proteins of the invention comprise the mature form of HSA fused to either the N- or C-terminus of the mature form of a therapeutic protein or portions thereof (e.g., the mature form of a therapeutic protein listed in Table 1, or the mature form of a therapeutic protein shown in Table 2 as SEQ ID NO:Z). In one embodiment of the invention, albumin fusion proteins of the invention further comprise a signal sequence which directs the nascent fusion polypeptide in the secretory pathways of the host used for expression. In a preferred embodiment, the signal peptide encoded by the signal sequence is removed, and the mature albumin fusion protein is secreted directly into the culture medium. Albumin fusion proteins of the invention preferably comprise heterologous signal sequences (e.g., the non-native signal sequence of a particular therapeutic protein) including, but not limited to, MAF, INV, Ig, Fibulin B, Clusterin, Insulin-Like Growth Factor Binding Protein 4, variant HSA leader sequences including, but not limited to, a chimeric HSA/MAF leader sequence, or other heterologous signal sequences known in the art. Especially preferred are those signal sequence listed in Table 2 and/or the signal sequence listed in the "Expression of Fusion Proteins" and/or "Additional Methods of Recombinant and Synthetic Production of Albumin Fusion Proteins" section of the specification, above. In preferred embodiments, the fusion proteins of the invention further comprise an N-terminal methionine residue. Polynucleotides encoding these polypeptides, including fragments and/or variants, are also encompassed by the invention.

[0912] Albumin fusion proteins from mammalian cell-line supernatants are purified according to different protocols depending on the expression system used.

Purification from CHO and 293T cell-lines.

[0913] Purification of an albumin fusion protein from CHO cell supernatant or from transiently transfected 293T cell supernatant may involve initial capture with an anionic HQ resin using a sodium phosphate buffer and a phosphate gradient elution, followed by affinity chromatography on a Blue Sepharose FF column using a salt gradient elution. Blue Sepharose FF removes the main BSA/fetuin contaminants. Further purification over the Poros PI 50 resin with a phosphate gradient may remove and lower endotoxin contamination as well as concentrate the albumin fusion protein.

Purification from NS0 cell-line.

[0914] Purification of an albumin-fusion protein from NS0 cell supernatant may involve Q-Sepharose anion exchange chromatography, followed by SP-sepharose purification with a step elution, followed by Phenyl-650M purification with a step elution, and, ultimately, diafiltration.

[0915] The purified protein may then be formulated by buffer exchange.

EXAMPLE 9: Bacterial Expression of an Albumin Fusion Protein.

[0916] A polynucleotide encoding an albumin fusion protein of the present invention comprising a bacterial signal sequence is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, to synthesize insertion fragments. The primers used to amplify the polynucleotide encoding insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

[0917] The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

[0918] Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 ($O.D.^{600}$) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

[0919] Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl or preferably in 8 M urea and concentrations greater than 0.14 M 2-mercaptoethanol by stirring for 3-4 hours at 4°C (see, e.g., Burton et al., Eur. J. Biochem. *179*:379-387 (1989)). The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

[0920] Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8. The column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

[0921] The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. Exemplary conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM immidazole. Immidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4°C or frozen at -80°C.

[0922] In addition to the above expression vector, the present invention further includes an expression vector, called pHE4a (ATCC Accession Number 209645, deposited on February 25, 1998) which contains phage operator and promoter elements operatively linked to a polynucleotide encoding an albumin fusion protein of the present invention, called pHE4a. (ATCC Accession Number 209645, deposited on February 25, 1998.) This vector contains: 1) a neomycinphosphotransferase gene as a selection marker, 2) an E. coli origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (lacIq). The origin of replication (oriC) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter and operator sequences are made synthetically.

[0923] DNA can be inserted into the pHE4a by restricting the vector with NdeI and XbaI, BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to PCR protocols described herein or otherwise known in the art, using PCR primers having restriction sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

[0924] The engineered vector may be substituted in the above protocol to express protein in a bacterial system.

EXAMPLE 10: Isolation of a Selected cDNA Clone From the Deposited Sample.

[0925] Many of the albumin fusion constructs of the invention have been deposited with the ATCC as shown in Table 3. The albumin fusion constructs may comprise any one of the following expression vectors: the yeast *S. cerevisiae* expression vector pSAC35, the mammalian expression vector pC4, or the mammalian expression vector pSE12.1.

[0926] pSAC35 (Sleep *et al.*, 1990, Biotechnology 8:42), pC4 (ATCC Accession No. 209646; Cullen *et al.*, Molecular and Cellular Biology, 438-447 (1985); Boshart *et al.*, Cell 41: 521-530 (1985)), and pSE12.1 (Lonza Biologics, Inc.; Stephens and Cockett, Nucl. Acids Res. 17: 7110 (1989); International Publication #WO89/01036; Murphy *et al.*, Biochem J. 227: 277-279 (1991); Bebbington *et al.*, Bio/Technology 10:169-175

(1992); US patent US 5,122,464; International Publication #WO86/05807) vectors comprise an ampicillin resistance gene for growth in bacterial cells. These vectors and/or an albumin fusion construct comprising them can be transformed into an *E. coli* strain such as Stratagene XL-1 Blue (Stratagene Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037) using techniques described in the art such as Hanahan, spread onto Luria-Broth agar plates containing 100 µg/mL ampicillin, and grown overnight at 37 °C.

[0927] The deposited material in the sample assigned the ATCC Deposit Number cited in Table 3 for any given albumin fusion construct also may contain one or more additional albumin fusion constructs, each encoding different albumin fusion proteins. Thus, deposits sharing the same ATCC Deposit Number contain at least an albumin fusion construct identified in the corresponding row of Table 3.

[0928] Two approaches can be used to isolate a particular albumin fusion construct from the deposited sample of plasmid DNAs cited for that albumin fusion construct in Table 3.

Method 1: Screening

[0929] First, an albumin fusion construct may be directly isolated by screening the sample of deposited plasmid DNAs using a polynucleotide probe corresponding to SEQ ID NO:X for an individual construct ID number in Table 1, using methods known in the art. For example, a specific polynucleotide with 30-40 nucleotides may be synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide can be labeled, for instance, with ^{32}P-γ-ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY (1982)). The albumin fusion construct from a given ATCC deposit is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using Nylon membranes according to routine methods for bacterial colony screening (e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

Method 2: PCR

[0930] Alternatively, DNA encoding a given albumin fusion protein may be amplified from a sample of a deposited albumin fusion construct with SEQ ID NO:X, for example, by using two primers of 17-20 nucleotides that hybridize to the deposited albumin fusion construct 5' and 3' to the DNA encoding a given albumin fusion protein. The polymerase chain reaction is carried out under routine conditions, for instance, in 25 µl of reaction mixture with 0.5 ug of the above cDNA template. A convenient reaction mixture is 1.5-5 mM $MgCl_2$, 0.01% (w/v) gelatin, 20 µM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94°C for 1 min; annealing at 55°C for 1 min; elongation at 72°C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

[0931] Several methods are available for the identification of the 5' or 3' non-coding portions of a gene which may not be present in the deposited clone. These methods include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE" protocols which are known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., Nucleic Acids Res., 21(7):1683-1684 (1993)).

[0932] Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5' portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

[0933] This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

[0934] This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the desired gene.

EXAMPLE 11: Multifusion Proteins

[0935] The albumin fusion proteins (e.g., containing a Therapeutic protein (or fragment or variant thereof) fused to albumin (or a fragment or variant thereof)) may additionally be fused to other proteins to generate "multifusion proteins". These multifusion proteins can be used for a variety of applications. For example, fusion of the albumin fusion proteins of the invention to His-tag, HA-tag, protein A, IgG domains, and maltose

binding protein facilitates purification. (see e.g. EP A 394,827; Traunecker et al., Nature 331:84-86 (1988)). Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of an albumin fusion protein. Furthermore, the fusion of additional protein sequences to the albumin fusion proteins of the invention may further increase the solubility and/or stability of the fusion protein. The fusion proteins described above can be made using or routinely modifying techniques known in the art and/or by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule.

[0936] Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian or yeast expression vector.

[0937] For example, if pC4 (ATCC Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and a polynucleotide encoding an albumin fusion protein of the present invention (generated and isolated using techniques known in the art), is ligated into this BamHI site. Note that the polynucleotide encoding the fusion protein of the invention is cloned without a stop codon, otherwise a Fc containing fusion protein will not be produced.

[0938] If the naturally occurring signal sequence is used to produce the albumin fusion protein of the present invention, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., International Publication No. WO 96/34891.)

[0939] Human IgG Fc region:

GGGATCCGGAGCCCAAATCTTCTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAATTCGAGGGTGCACCGTCAGTCTTCC
TCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACTCCTGAGGTCACATGCGTGGTGGTGGACGTAAGCCACGAAGACC
CTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACG
TACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTC
CCAACCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGA
GCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGC
AGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGA
GCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGT
CTCCGGGTAAATGAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ ID NO:52)

EXAMPLE 12: Production of an Antibody from an Albumin Fusion Protein.

Hybridoma Technology

[0940] Antibodies that bind the albumin fusion proteins of the present invention and portions of the albumin fusion proteins of the present invention (e.g., the Therapeutic protein portion or albumin portion of the fusion protein) can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, a preparation of an albumin fusion protein of the invention or a portion of an albumin fusion protein of the invention is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

[0941] Monoclonal antibodies specific for an albumin fusion protein of the invention, or a portion of an albumin fusion protein of the invention, are prepared using hybridoma technology (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol. 6:511 (1976); Kohler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized with an albumin fusion protein of the invention, or a portion of an albumin fusion protein of the invention. The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding an albumin fusion protein of the invention, or a portion of an albumin fusion protein of the invention.

[0942] Alternatively, additional antibodies capable of binding to an albumin fusion protein of the invention, or a portion of an albumin fusion protein of the invention can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the an albumin fusion protein of the invention (or portion of an albumin fusion protein of the invention) -specific antibody can be blocked by the fusion protein of the invention, or a portion of an albumin fusion protein of the invention. Such antibodies comprise anti-idiotypic antibodies to the fusion protein of the invention (or portion of an albumin fusion protein of the invention) -specific antibody and are used to immunize an animal to induce formation

of further fusion protein of the invention (or portion of an albumin fusion protein of the invention) -specific antibodies.

[0943] For *in vivo* use of antibodies in humans, an antibody is "humanized". Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized antibodies are known in the art and are discussed herein. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., International Publication No. WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985)).

[0944] ***Isolation Of Antibody Fragments Directed Against an albumin fusion protein of the invention, or a portion of an albumin fusion protein of the Invention From A Library Of scFvs.*** Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against an albumin fusion protein of the invention, or a portion of an albumin fusion protein of the invention, to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein by reference in its entirety).

[0945] ***Rescue of the Library.*** A library of scFvs is constructed from the RNA of human PBLs as described in International Publication No. WO 92/01047. To rescue phage displaying antibody fragments, approximately 10^9 *E. coli* harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 μg/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-AMP-GLU, 2 x 108 TU of delta gene 3 helper (M13 delta gene III, see International Publication No. WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY containing 100 μg/ml ampicillin and 50 ug/ml kanamycin and grown overnight. Phage are prepared as described in International Publication No. WO 92/01047.

[0946] M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100 μg ampicillin/ml and 25 μg kanamycin/ml (2xTY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 μm filter (Minisart NML; Sartorius) to give a final concentration of approximately 10^{13} transducing units/ml (ampicillin-resistant clones).

[0947] ***Panning of the Library.*** Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 μg/ml or 10 μg/ml of an albumin fusion protein of the invention, or a portion of an albumin fusion protein of the invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately 10^{13} TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The E. coli are then plated on TYE plates containing 1% glucose and 100 μg/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

[0948] ***Characterization of Binders.*** Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 pg/ml of an albumin fusion protein of the invention, or a portion of an albumin fusion protein of the invention, in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see, e.g., International Publication No. WO 92/01047) and then by sequencing. These ELISA positive clones may also be further characterized by techniques known in the art, such as, for example, epitope mapping, binding affinity, receptor signal transduction, ability to block or competitively inhibit antibody/antigen binding, and competitive agonistic or antagonistic activity.

EXAMPLE 13: ^{3}H-2-Deoxyglucose Uptake Assay.

[0949] Adipose, skeletal muscle, and liver are insulin-sensitive tissues. Insulin can stimulate glucose uptake/transport into these tissues. In the case of adipose and skeletal muscle, insulin initiates the signal transduction that eventually leads to the translocation of the glucose transporter 4 molecule, GLUT4, from a specialized intracellular compartment to the cell surface. Once on the cell surface, GLUT4 allows for glucose uptake/transport.

^{3}H-2-Deoxyglucose Uptake

[0950] A number of adipose and muscle related cell-lines can be used to test for glucose uptake/transport activity in the absence or presence of a combination of any one or more of the therapeutic drugs listed for the treatment of diabetes mellitus. In particular, the 3T3-L1 murine fibroblast cells and the L6 murine skeletal muscle cells can be differentiated into 3T3-L1 adipocytes and into myotubes, respectively, to serve as appropriate *in vitro* models for the [^{3}H]-2-deoxyglucose uptake assay (Urso et al., J Biol Chem, 274(43): 30864-73 (1999); Wang et al., J Mol Endocrinol, 19(3):

241-8 (1997); Hampel et al., J Membr Biol, 169 (1): 45-53 (1999); Tsakiridis et al., Endocrinology, 136(10): 4315-22 (1995)). Briefly, 2 x 10^5 cells/100 µL of adipocytes or differentiated L6 cells are transferred to 96-well Tissue-Culture, "TC", treated, i.e., coated with 50 µg/mL of poly-L-lysine, plates in post-differentiation medium and are incubated overnight at 37 °C in 5% CO_2. The cells are first washed once with serum free low glucose DMEM medium and are then starved with 100 µL/well of the same medium and with 100 µL/well of either buffer or of a combination of any one or more of the therapeutic drugs listed for the treatment of diabetes mellitus, for example, increasing concentrations of 1 nM, 10 nM, and 100 nM of the therapeutics of the subject invention (e.g., specific fusions disclosed as SEQ ID NO:Y and fragments and variants thereof) for 16 hours at 37 °C in the absence or presence of 1 nM insulin. The plates are washed three times with 100 µL/well of HEPES buffered saline. Insulin is added at 1 nM in HEPES buffered saline for 30 min at 37 °C in the presence of 10 µM labeled [^{3}H]-2-deoxyglucose (Amersham, #TRK672) and 10 µM unlabeled 2-deoxyglucose (SIGMA, D-3179). As control, the same conditions are carried out except in the absence of insulin. A final concentration of 10 µM cytochalasin B (SIGMA, C6762) is added at 100 µL/well in a separate well to measure the non-specific uptake. The cells are washed three times with HEPES buffered saline. Labeled, i.e., 10 µM of [^{3}H]-2-deoxyglucose, and unlabeled, i.e., 10 µM of 2-deoxyglucose, are added for 10 minutes at room temperature. The cells are washed three times with cold Phosphate Buffered *Saline* ine, "PBS". The cells are lysed upon the addition of 150 µL/well of 0.2 N NaOH and subsequent incubation with shaking for 20 minutes at room temperature. Samples are then transferred to a scintillation vial to which is added 5 mL of scintillation fluid. The vials are counted in a Beta-Scintillation counter. Uptake in duplicate conditions, the difference being the absence or presence of insulin, is determined with the following equation: [(Insulin counts per minute "cpm" – Non-Specific cpm)/(No Insulin cpm – Non-Specific cpm)]. Average responses fall within the limits of about 5-fold and 3-fold that of controls for adipocytes and myotubes, respectively.

Differentiation of Cells

[0951] The cells are allowed to become fully confluent in a T-75 cm^2 flask. The medium is removed and replaced with 25 mL of pre-differentiation medium for 48 hours. The cells are incubated at 37 °C, in 5% CO_2, 85% humidity. After 48 hours, the pre-differentiation medium is removed and replaced with 25 mL differentiation medium for 48 hours. The cells are again incubated at 37 °C, in 5% CO_2, 85% humidity. After 48 hours, the medium is removed and replaced with 30 mL post-differentiation medium. Post-differentiation medium is maintained for 14-20 days or until complete differentiation is achieved. The medium is changed every 2-3 days. Human adipocytes can be purchased from Zen-Bio, INC (# SA-1096).

EXAMPLE 14: In vitro Assay of [^{3}H]-Thymidine Incorporation into Pancreatic Cell-lines.

[0952] It has recently been shown that GLP-1 induces differentiation of the rat pancreatic ductal epithelial cell-line ARIP in a time- and dose-dependent manner which is associated with an increase in Islet Duodenal Homeobox-1 (IDX-1) and insulin mRNA levels (Hui et al., 2001, Diabetes, 50(4): 785-96). The IDX-1 in turn increases mRNA levels of the GLP-1 receptor.

Cells Types Tested

[0953] RIN-M cells: These cells are available from the American Type Tissue Culture Collection (ATCC Cell Line Number CRL-2057). The RIN-M cell line was derived from a radiation induced transplantable rat islet cell tumor. The line was established from a nude mouse xenograft of the tumor. The cells produce and secrete islet polypeptide hormones, and produce L-dopa decarboxylase (a marker for cells having amine precursor uptake and decarboxylation, or APUD, activity).

[0954] ARIP cells: These are pancreatic exocrine cells of epithelial morphology available from the American Type Tissue Culture Collection (ATCC Cell Line Number CRL-1674). See also, references: Jessop, N.W. and Hay, R.J., "Characteristics of two rat pancreatic exocrine cell lines derived from transplantable tumors," In Vitro 16: 212, (1980); Cockell, M. et al., "Identification of a cell-specific DNA-binding activity that interacts with a transcriptional activator of genes expressed in the acinar pancreas," Mol. Cell. Biol. 9: 2464-2476, (1989); Roux, E., et al. "The cell-specific transcription factor PTF1 contains two different subunits that interact with the DNA" Genes Dev. 3: 1613-1624, (1989); and, Hui, H., et al., "Glucagon-like peptide 1 induces differentiation of islet duodenal homeobox-1-positive pancreatic ductal cells into insulin-secreting cells," Diabetes 50: 785-796 (2001).

Preparation of Cells

[0955] The RIN-M cell-line is grown in RPMI 1640 medium (Hyclone, #SH300027.01) with 10% fetal bovine serum (HyClone, #SH30088.03) and is subcultured every 6 to 8 days at a ratio of 1:3 to 1:6. The medium is changed every 3 to 4 days.

[0956] The ARIP (ATCC #CRL-1674) cell-line is grown in Ham's F12K medium (ATCC, #30-2004) with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 10% fetal bovine serum. The ARIP cell-line is subcultured at a ratio of 1:3 to 1:6 twice per week. The medium is changed every 3 to 4 days.

Assay Protocol

[0957] The cells are seeded at 4000 cells/well in 96-well plates and cultured for 48 to 72 hours to 30% confluence. The cells are switched to serum-free media at 100 µL/well. After incubation for 48-72 hours, serum and/or the therapeutics of the subject invention (e.g., albumin fusion proteins of the invention and fragments and variants thereof) are added to the well. Incubation persists for an additional 36 hours. [^{3}H]-Thymidine

(5-20 Ci/mmol) (Amersham Pharmacia, #TRK120) is diluted to 1 microCuries/5 microliters. After the 36 hour incubation, 5 microliters is added per well for a further 24 hours. The reaction is terminated by washing the cells gently with cold Phosphate-Buffered *Saline*, "PBS", once. The cells are then fixed with 100 microliters of 10% ice cold TCA for 15 min at 4 °C. The PBS is removed and 200 microliters of 0.2 N NaOH is added. The plates are incubated for 1 hour at room temperature with shaking. The solution is transferred to a scintillation vial and 5 mL of scintillation fluid compatible with aqueous solutions is added and mixed vigorously. The vials are counted in a beta scintillation counter. As negative control, only buffer is used. As a positive control fetal calf serum is used.

EXAMPLE 15: Assays for Glycosuria.

[0958] Glycosuria (i.e., excess sugar in the urine), can be readily assayed to provide an index of the disease state of diabetes mellitus. Excess urine in a patient sample as compared with a normal patient sample is symptomatic of IDDM and NIDDM. Efficacy of treatment of such a patient having IDDM and NIDDM is indicated by a resulting decrease in the amount of excess glucose in the urine. In a preferred embodiment for IDDM and NIDDM monitoring, urine samples from patients are assayed for the presence of glucose using techniques known in the art. Glycosuria in humans is defined by a urinary glucose concentration exceeding 100 mg per 100 ml. Excess sugar levels in those patients exhibiting glycosuria can be measured even more precisely by obtaining blood samples and assaying serum glucose.

EXAMPLE 16: Assays Detecting Stimulation or Inhibition of B cell Proliferation and Differentiation.

[0959] Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations.

[0960] One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

[0961] In Vitro Assay- Albumin fusion proteins of the invention (including fusion proteins containing fragments or variants of Therapeutic proteins and/or albumin or fragments or variants of albumin) can be assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of an albumin fusion protein of the invention on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay *in which purified tonsillar B cells are cultured in the presence of either formalin-fixed Staphylococcus aureus* Cowan I (SAC) *or immobilized anti-*human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220).

[0962] Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added 10^5 B-cells suspended in culture medium (RPMI 1640 containing 10% FBS, 5 X 10^{-5}M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and 10^{-5} dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with 3H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

[0963] *In vivo* Assay- BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of an albumin fusion protein of the invention (including fusion proteins containing fragments or variants of Therapeutic proteins and/or albumin or fragments or variants of albumin). Mice receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal spleens and spleens treated with the albumin fusion protein of the invention identify the results of the activity of the fusion protein on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

[0964] Flow cytometric analyses of the spleens from mice treated with the albumin fusion protein is used to indicate whether the albumin fusion protein specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice.

[0965] Likewise, a predicted consequence of increased mature B-cell representation *in vivo* is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared between buffer and fusion protein treated mice.

EXAMPLE 17: T Cell Proliferation Assay.

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[0966] A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of ^{3}H-thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100 µl/well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control mAb (B33.1) overnight at 4 degrees C (1 µg/ml in .05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells (5 x 10^4/well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of an albumin fusion protein of the invention (including fusion proteins containing fragments or variants of Therapeutic proteins and/or albumin or fragments or variants of albumin) (total volume 200 ul). Relevant protein buffer and medium alone are controls. After 48 hr. culture at 37 degrees C, plates are spun for 2 min. at 1000 rpm and 100 µl of supernatant is removed and stored –20 degrees C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100 ul of medium containing 0.5 uCi of ^{3}H-thymidine and cultured at 37 degrees C for 18-24 hr. Wells are harvested and incorporation of ^{3}H-thymidine used as a measure of proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative control for the effects of fusion proteins of the invention.

EXAMPLE 18: Effect of Fusion Proteins of the Invention on the Expression of MHC Class II, Costimulatory and Adhesion Molecules and Cell Differentiation of Monocytes and Monocyte-Derived Human Dendritic Cells.

[0967] Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as TNF-α, causes a rapid change in surface phenotype (increased expression of MHC class I and II, costimulatory and adhesion molecules, downregulation of FCγRII, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional maturation of the dendritic cells.

[0968] FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of an albumin fusion protein of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

[0969] Effect on the production of cytokines. Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune responses. IL-12 strongly influences the development of Th1 helper T-cell immune response, and induces cytotoxic T and NK cell function. An ELISA is used to measure the IL-12 release as follows. Dendritic cells (10^6/ml) are treated with increasing concentrations of an albumin fusion protein of the invention for 24 hours. LPS (100 ng/ml) is added to the cell culture as positive control. Supernatants from the cell cultures are then collected and analyzed for IL-12 content using commercial ELISA kit (e.g., R & D Systems (Minneapolis, MN)). The standard protocols provided with the kits are used.

[0970] Effect on the expression of MHC Class II, costimulatory and adhesion molecules. Three major families of cell surface antigens can be identified on monocytes: adhesion molecules, molecules involved in antigen presentation, and Fc receptor. Modulation of the expression of MHC class II antigens and other costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T cell activation. Increased expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

[0971] FACS analysis is used to examine the surface antigens as follows. Monocytes are treated 1-5 days with increasing concentrations of an albumin fusion protein of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

[0972] Monocyte activation and/or increased survival. Assays for molecules that activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine whether a molecule of the invention functions as an inhibitor or activator of monocytes. Albumin fusion proteins of the invention can be screened using the three assays described below. For each of these assays, Peripheral blood mononuclear cells (PBMC) are purified from single donor leukopacks (American Red Cross, Baltimore, MD) by centrifugation through a Histopaque gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

[0973] Monocyte Survival Assay. Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results from internally regulated processes (apoptosis). Addition to the culture of activating factors, such as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying concentrations of the fusion protein to be tested. Cells are suspended at a concentration of 2 x 10^6/ml in PBS containing PI at a final concentration of 5 µg/ml, and then incubated at room temperature for 5 minutes before FACScan analysis. PI

uptake has been demonstrated to increase with DNA fragmentation in this experimental paradigm.

[0974] Effect on cytokine release. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows. Human monocytes are incubated at a density of 5×10^5 cells/ml with increasing concentrations of an albumin fusion protein of the invention and under the same conditions, but in the absence of the fusion protein. For IL-12 production, the cells are primed overnight with IFN (100 U/ml) in the presence of the fusion protein. LPS (10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until use. Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e.g., R & D Systems (Minneapolis, MN)) and applying the standard protocols provided with the kit.

[0975] Oxidative burst. Purified monocytes are plated in 96-w plate at $2\text{-}1 \times 10^5$ cell/well. Increasing concentrations of an albumin fusion protein of the invention are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is removed from the wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRPO) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20 µl 1N NaOH per well. The absorbance is read at 610 nm. To calculate the amount of H_2O_2 produced by the macrophages, a standard curve of a H_2O_2 solution of known molarity is performed for each experiment.

EXAMPLE 19: The Effect of Albumin Fusion Proteins of the Invention on the Growth of Vascular Endothelial Cells.

[0976] On day 1, human umbilical vein endothelial cells (HUVEC) are seeded at $2\text{-}5 \times 10^4$ cells/35 mm dish density in M199 medium containing 4% fetal bovine serum (FBS), 16 units/ml heparin, and 50 units/ml endothelial cell growth supplements (ECGS, Biotechnique, Inc.). On day 2, the medium is replaced with M199 containing 10% FBS, 8 units/ml heparin. An albumin fusion protein of the invention, and positive controls, such as VEGF and basic FGF (bFGF) are added, at varying concentrations. On days 4 and 6, the medium is replaced. On day 8, cell number is determined with a Coulter Counter.

[0977] An increase in the number of HUVEC cells indicates that the fusion protein may proliferate vascular endothelial cells, while a decrease in the number of HUVEC cells indicates that the fusion protein inhibits vascular endothelial cells.

EXAMPLE 20: Rat Corneal Wound Healing Model.

[0978] This animal model shows the effect of an albumin fusion protein of the invention on neovascularization. The experimental protocol includes:

Making a 1-1.5 mm long incision from the center of cornea into the stromal layer.

Inserting a spatula below the lip of the incision facing the outer corner of the eye.

Making a pocket (its base is 1-1.5 mm form the edge of the eye).

Positioning a pellet, containing 50ng- 5ug of an albumin fusion protein of the invention, within the pocket.

Treatment with an an albumin fusion protein of the invention can also be applied topically to the corneal wounds in a dosage range of 20mg - 500mg (daily treatment for five days).

EXAMPLE 21: Diabetic Mouse and Glucocorticoid-Impaired Wound Healing Models.

Diabetic db+/db+ Mouse Model.

[0979] To demonstrate that an albumin fusion protein of the invention accelerates the healing process, the genetically diabetic mouse model of wound healing is used. The full thickness wound healing model in the db+/db+ mouse is a well characterized, clinically relevant and reproducible model of impaired wound healing. Healing of the diabetic wound is dependent on formation of granulation tissue and re-epithelialization rather than contraction (Gartner, *M.H. et al., J. Surg. Res. 52*:389 (1992); Greenhalgh, *D.G. et al., Am. J. Pathol. 136*:1235 (1990)).

[0980] The diabetic animals have many of the characteristic features observed in Type II diabetes mellitus. Homozygous (db+/db+) mice are obese in comparison to their normal heterozygous (db+/+m) littermates. Mutant diabetic (db+/db+) mice have a single autosomal recessive mutation on chromosome 4 (db+) (Coleman *et al. Proc. Natl. Acad. Sci. USA 77*:283-293 (1982)). Animals show polyphagia, polydipsia and polyuria. Mutant diabetic mice (db+/db+) have elevated blood glucose, increased or normal insulin levels, and suppressed cell-mediated immunity (Mandel *et al., J. Immunol. 120*:1375 (1978); Debray-Sachs, *M. et al., Clin. Exp. Immunol. 51(1)*:1-7 (1983); Leiter *et al., Am. J. of Pathol. 114*:46-55 (1985)). Peripheral neuropathy, myocardial complications, and microvascular lesions, basement membrane thickening and glomerular filtration abnormalities have been described in these animals (Norido, *F. et al., Exp. Neurol. 83(2)*:221-232 (1984); Robertson *et al., Diabetes 29(1)*:60-67 (1980); Giacomelli *et al., Lab Invest. 40(4)*:460-473 (1979); Coleman, D.L., *Diabetes 31 (Suppl)*:1-6 (1982)). These homozygous diabetic mice develop hyperglycemia that is resistant to insulin analogous to human type II diabetes (Mandel *et al., J. Immunol. 120*:1375-1377 (1978)).

[0981] The characteristics observed in these animals suggests that healing in this model may be similar to the healing observed in human diabetes (Greenhalgh, *et al., Am. J. of Pathol. 136*:1235-1246 (1990)).

[0982] Genetically diabetic female C57BL/KsJ (db+/db+) mice and their non-diabetic (db+/+m) heterozygous littermates are used in this study (Jackson Laboratories). The animals are purchased at 6 weeks of age and are 8 weeks old at the beginning of the study. Animals are individually

housed and received food and water ad libitum. All manipulations are performed using aseptic techniques. The experiments are conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

[0983] Wounding protocol is performed according to previously reported methods (Tsuboi, R. and Rifkin, D.B., *J. Exp. Med. 172*:245-251 (1990)). Briefly, on the day of wounding, animals are anesthetized with an intraperitoneal injection of Avertin (0.01 mg/mL), 2,2,2-tribromoethanol and 2-methyl-2-butanol dissolved in deionized water. The dorsal region of the animal is shaved and the skin washed with 70% ethanol solution and iodine. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is then created using a Keyes tissue punch. Immediately following wounding, the surrounding skin is gently stretched to eliminate wound expansion. The wounds are left open for the duration of the experiment. Application of the treatment is given topically for 5 consecutive days commencing on the day of wounding. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

[0984] Wounds are visually examined and photographed at a fixed distance at the day of surgery and at two day intervals thereafter. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

[0985] An albumin fusion protein of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

[0986] Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology and immunohistochemistry. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

[0987] Three groups of 10 animals each (5 diabetic and 5 non-diabetic controls) are evaluated: 1) Vehicle placebo control, 2) untreated group, and 3) treated group.

[0988] Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total square area of the wound. Contraction is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm^2, the corresponding size of the dermal punch. Calculations are made using the following formula:

a. [Open area on day 8] - [Open area on day 1] / [Open area on day 1]

[0989] Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using a Reichert-Jung microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds are used to assess whether the healing process and the morphologic appearance of the repaired skin is altered by treatment with an albumin fusion protein of the invention. This assessment included verification of the presence of cell accumulation, inflammatory cells, capillaries, fibroblasts, re-epithelialization and epidermal maturity (Greenhalgh, D.G. *et al., Am. J. Pathol. 136*:1235 (1990)). A calibrated lens micrometer is used by a blinded observer.

[0990] Tissue sections are also stained immunohistochemically with a polyclonal rabbit anti-human keratin antibody using ABC Elite detection system. Human skin is used as a positive tissue control while non-immune IgG is used as a negative control. Keratinocyte growth is determined by evaluating the extent of reepithelialization of the wound using a calibrated lens micrometer.

[0991] Proliferating cell nuclear antigen/cyclin (PCNA) in skin specimens is demonstrated by using anti-PCNA antibody (1:50) with an ABC Elite detection system. Human colon cancer served as a positive tissue control and human brain tissue is used as a negative tissue control. Each specimen included a section with omission of the primary antibody and substitution with non-immune mouse IgG. Ranking of these sections is based on the extent of proliferation on a scale of 0-8, the lower side of the scale reflecting slight proliferation to the higher side reflecting intense proliferation.

[0992] Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

Steroid Impaired Rat Model

[0993] The inhibition of wound healing by steroids has been well documented in various *in vitro* and *in vivo* systems (Wahl, Glucocorticoids and Wound healing. In: Anti-Inflammatory Steroid Action: Basic and Clinical Aspects. 280-302 (1989); Wahl *et al., J. Immunol. 115*: 476-481 (1975); Werb *et al., J. Exp. Med. 147*:1684-1694 (1978)). Glucocorticoids retard wound healing by inhibiting angiogenesis, decreasing vascular permeability (Ebert *et al., An. Intern. Med. 37*:701-705 (1952)), fibroblast proliferation, and collagen synthesis (Beck *et al., Growth Factors. 5:* 295-304 (1991); Haynes *et al., J. Clin. Invest. 61:* 703-797 (1978)) and producing a transient reduction of circulating monocytes (Haynes et al., *J. Clin. Invest. 61:* 703-797 (1978); Wahl, "Glucocorticoids and wound healing", *In:* Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989)). The systemic administration of steroids to impaired wound healing is a well establish phenomenon in rats (Beck *et al., Growth Factors. 5:* 295-304 (1991); Haynes *et al., J. Clin. Invest. 61:* 703-797 (1978); Wahl, "Glucocorticoids and wound healing", *in:* Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989); Pierce *et al., Proc. Natl. Acad. Sci. USA 86*: 2229-2233 (1989)).

[0994] To demonstrate that an albumin fusion protein of the invention can accelerate the healing process, the effects of multiple topical

applications of the fusion protein on full thickness excisional skin wounds in rats in which healing has been impaired by the systemic administration of methylprednisolone is assessed.

[0995] Young adult male Sprague Dawley rats weighing 250-300 g (Charles River Laboratories) are used in this example. The animals are purchased at 8 weeks of age and are 9 weeks old at the beginning of the study. The healing response of rats is impaired by the systemic administration of methylprednisolone (17mg/kg/rat intramuscularly) at the time of wounding. Animals are individually housed and received food and water *ad libitum*. All manipulations are performed using aseptic techniques. This study is conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

[0996] The wounding protocol is followed according to that described above. On the day of wounding, animals are anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The dorsal region of the animal is shaved and the skin washed with 70% ethanol and iodine solutions. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is created using a Keyes tissue punch. The wounds are left open for the duration of the experiment. Applications of the testing materials are given topically once a day for 7 consecutive days commencing on the day of wounding and subsequent to methylprednisolone administration. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

[0997] Wounds are visually examined and photographed at a fixed distance at the day of wounding and at the end of treatment. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jamesson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

[0998] The fusion protein of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

[0999] Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

[1000] Three groups of 10 animals each (5 with methylprednisolone and 5 without glucocorticoid) are evaluated: 1) Untreated group 2) Vehicle placebo control 3) treated groups.

[1001] Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total area of the wound. Closure is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm^2, the corresponding size of the dermal punch. Calculations are made using the following formula:

b. [Open area on day 8] - [Open area on day 1] / [Open area on day 1]

[1002] Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using an Olympus microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds allows assessment of whether the healing process and the morphologic appearance of the repaired skin is improved by treatment with an albumin fusion protein of the invention. A calibrated lens micrometer is used by a blinded observer to determine the distance of the wound gap.

[1003] Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

EXAMPLE 22: Lymphedema Animal Model.

[1004] The purpose of this experimental approach is to create an appropriate and consistent lymphedema model for testing the therapeutic effects of an albumin fusion protein of the invention in lymphangiogenesis and re-establishment of the lymphatic circulatory system in the rat hind limb. Effectiveness is measured by swelling volume of the affected limb, quantification of the amount of lymphatic vasculature, total blood plasma protein, and histopathology. Acute lymphedema is observed for 7-10 days. Perhaps more importantly, the chronic progress of the edema is followed for up to 3-4 weeks.

[1005] Prior to beginning surgery, blood sample is drawn for protein concentration analysis. Male rats weighing approximately ~350g are dosed with Pentobarbital. Subsequently, the right legs are shaved from knee to hip. The shaved area is swabbed with gauze soaked in 70% EtOH. Blood is drawn for serum total protein testing. Circumference and volumetric measurements are made prior to injecting dye into paws after marking 2 measurement levels (0.5 cm above heel, at mid-pt of dorsal paw). The intradermal dorsum of both right and left paws are injected with 0.05 ml of 1% Evan's Blue. Circumference and volumetric measurements are then made following injection of dye into paws.

[1006] Using the knee joint as a landmark, a mid-leg inguinal incision is made circumferentially allowing the femoral vessels to be located. Forceps and hemostats are used to dissect and separate the skin flaps. After locating the femoral vessels, the lymphatic vessel that runs along side and underneath the vessel(s) is located. The main lymphatic vessels in this area are then electrically coagulated or suture ligated.

[1007] Using a microscope, muscles in back of the leg (near the semitendinosis and adductors) are bluntly dissected. The popliteal lymph node is then located. The 2 proximal and 2 distal lymphatic vessels and distal blood supply of the popliteal node are then ligated by suturing. The popliteal lymph node, and any accompanying adipose tissue, is then removed by cutting connective tissues.

[1008] Care is taken to control any mild bleeding resulting from this procedure. After lymphatics are occluded, the skin flaps are sealed by

using liquid skin (Vetbond) (AJ Buck). The separated skin edges are sealed to the underlying muscle tissue while leaving a gap of ~0.5 cm around the leg. Skin also may be anchored by suturing to underlying muscle when necessary.

[1009] To avoid infection, animals are housed individually with mesh (no bedding). Recovering animals are checked daily through the optimal edematous peak, which typically occurred by day 5-7. The plateau edematous peak are then observed. To evaluate the intensity of the lymphedema, the circumference and volumes of 2 designated places on each paw before operation and daily for 7 days are measured. The effect of plasma proteins on lymphedema is determined and whether protein analysis is a useful testing perimeter is also investigated. The weights of both control and edematous limbs are evaluated at 2 places. Analysis is performed in a blind manner.

[1010] Circumference Measurements: Under brief gas anesthetic to prevent limb movement, a cloth tape is used to measure limb circumference. Measurements are done at the ankle bone and dorsal paw by 2 different people and those 2 readings are averaged. Readings are taken from both control and edematous limbs.

[1011] Volumetric Measurements: On the day of surgery, animals are anesthetized with Pentobarbital and are tested prior to surgery. For daily volumetrics animals are under brief halothane anesthetic (rapid immobilization and quick recovery), and both legs are shaved and equally marked using waterproof marker on legs. Legs are first dipped in water, then dipped into instrument to each marked level then measured by Buxco edema software(Chen/Victor). Data is recorded by one person, while the other is dipping the limb to marked area.

[1012] Blood-plasma protein measurements: Blood is drawn, spun, and serum separated prior to surgery and then at conclusion for total protein and $Ca2^+$ comparison.

[1013] Limb Weight Comparison: After drawing blood, the animal is prepared for tissue collection. The limbs are amputated using a quillitine, then both experimental and control legs are cut at the ligature and weighed. A second weighing is done as the tibio-cacaneal joint is disarticulated and the foot is weighed.

[1014] Histological Preparations: The transverse muscle located behind the knee (popliteal) area is dissected and arranged in a metal mold, filled with freezeGel, dipped into cold methylbutane, placed into labeled sample bags at - 80EC until sectioning. Upon sectioning, the muscle is observed under fluorescent microscopy for lymphatics..

EXAMPLE 33: Suppression of TNF alpha-Induced Adhesion Molecule Expression by an Albumin Fusion Protein of the Invention.

[1015] The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

[1016] Tumor necrosis factor alpha (TNF-a), a potent proinflammatory cytokine, is a stimulator of all three CAMs on endothelial cells and may be involved in a wide variety of inflammatory responses, often resulting in a pathological outcome.

[1017] The potential of an albumin fusion protein of the invention to mediate a suppression of TNF-a induced CAM expression can be examined. A modified ELISA assay which uses ECs as a solid phase absorbent is employed to measure the amount of CAM expression on TNF-a treated ECs when co-stimulated with a member of the FGF family of proteins.

[1018] To perform the experiment, human umbilical vein endothelial cell (HUVEC) cultures are obtained from pooled cord harvests and maintained in growth medium (EGM-2; Clonetics, San Diego, CA) supplemented with 10% FCS and 1% penicillin/streptomycin in a 37 degree C humidified incubator containing 5% CO_2. HUVECs are seeded in 96-well plates at concentrations of 1×10^4 cells/well in EGM medium at 37 degree C for 18-24 hrs or until confluent. The monolayers are subsequently washed 3 times with a serum-free solution of RPMI-1640 supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin, and treated with a given cytokine and/or growth factor(s) for 24 h at 37 degree C. Following incubation, the cells are then evaluated for CAM expression.

[1019] Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well plate to confluence. Growth medium is removed from the cells and replaced with 90 ul of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 ul volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 µl of 0.1% paraformaldehyde-PBS(with Ca^{++} and Mg^{++}) is added to each well. Plates are held at 4°C for 30 min.

[1020] Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10 µl of diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 µg/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified

environment. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA.

[1021] Then add 20 µl of diluted ExtrAvidin-Alkaline Phosphotase (1:5,000 dilution) to each well and incubated at 37°C for 30 min. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA. 1 tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH 10.4). 100 µl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphotase in glycine buffer: 1:5,000 (10^{0}) > $10^{-0.5}$ > 10^{-1} > $10^{-1.5}$. 5 µl of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 µl of pNNP reagent must then be added to each of the standard wells. The plate must be incubated at 37°C for 4h. A volume of 50 µl of 3M NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

EXAMPLE 24: Construction of GAS Reporter Constructs

[1022] One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathway bind to gamma activation site "GAS" elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

[1023] GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in many cell types though it has been found in T helper class I, cells after treatment with IL-12. Stat5 was originally called mammary growth factor, but has been found at higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

[1024] The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

[1025] The Jaks are activated by a wide range of receptors summarized in the Table below. (Adapted from review by Schindler and Darnell, Ann. Rev. Biochem. 64:621-51 (1995)). A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b) Class 2 includes IFN-a, IFN-g, and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xaa-Trp-Ser (SEQ ID NO:53)).

[1026] Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway. Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the Jaks-STATs pathway (See Table 5, below). Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

Table 5

JAKs		STATS			GAS(elements) or ISRE	
Ligand	tyk2	Jak1	Jak2	Jak3		
IFN family						
IFN-a/B	+	+	-	-	1,2,3	ISRE
IFN-g		+	+	-	1	GAS (IRF1>Lys6>IFP)
Il-10	+	?	?	-	1,3	
gp130 family						
IL-6 (Pleiotropic)	+	+	+	?	1,3	GAS(IRF1>Lys6>IFP)
Il-11(Pleiotropic)	?	+	?	?	1,3	
OnM(Pleiotropic)	?	+	+	?	1,3	
LIF(Pleiotropic)	?	+	+	?	1,3	
CNTF(Pleiotropic)	-/+	+	+	?	1,3	
G-CSF(Pleiotropic)	?	+	?	?	1,3	
IL-12(Pleiotropic)	+	-	+	+	1,3	
g-C family						
IL-2 (lymphocytes)	-	+	-	+	1,3,5	GAS
IL-4 (lymph/myeloid)	-	+	-	+	6	GAS(IRF1=IFP>>Ly6)(IgH)
IL-7 (lymphocytes)	-	+	-	+	5	GAS
IL-9 (lymphocytes)	-	+	-	+	5	GAS
IL-13 (lymphocyte)	-	+	?	?	6	GAS
IL-15	?	+	?	+	5	GAS
gp140 family						

IL-3 (myeloid)	-	-	+	-	5	GAS(IRF1>IFP>>Ly6)
IL-5 (myeloid)	-	-	+	-	5	GAS
GM-CSF (myeloid)	-	-	+	-	5	GAS
Growth hormone family						
GH	?	-	+	-	5	
PRL	?	+/-	+	-	1,3,5	
EPO	?	-	+	-	5	GAS (B-CAS>IRF1=IFP>>Ly6)
Receptor Tyrosine Kinases						
EGF	?	+	+	-	1,3	GAS (IRF1)
PDGF	?	+	+	-	1,3	
CSF-1	?	+	+	-	1,3	GAS(not IRF1)

[1027] To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 27-29, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothnan et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an XhoI site. The sequence of the 5' primer is:

5':GCGCCTCGAGATTTCCCCGAAATCTAGATTTCCCCGAAATGATTTCCCCGAAATGATTTCCCCGAAATATCTGCCATCTCAATTAG: 3' (SEQ ID NO:54)

[1028] The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:55)

[1029] PCR amplification is performed using the SV40 promoter template present in the B-gal-promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence:

5':CTCGAGATTTCCCCGAAATCTAGATTTCCCCGAAATGATTTCCCCGAAATGATTTCCCCGAAATATCTGCCATCTCAATTAGTCAGC AACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTT TTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAA AAAGCTT:3' (SEQ ID NO:56)

[1030] With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

[1031] The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindIII and XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

[1032] Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using SalI and NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 27-29.

[1033] Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing EGR and NF-KB promoter sequences are described in Examples 27-31. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, Il-2/NFAT, or NF-KB/OAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

EXAMPLE 25: Assay for SEAP Activity.

[1034] As a reporter molecule for the assays described in examples disclosed herein, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

[1035] Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 ul of 2.5x dilution buffer into Optiplates containing 35 ul of a solution containing an albumin fusion protein of the invention. Seal the plates with a plastic sealer and incubate at 65 degree C for 30 min. Separate the Optiplates to avoid uneven heating.

[1036] Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 ml Assay Buffer

and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the Table below). Add 50 ul Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on a luminometer, thus one should treat 5 plates at each time and start the second set 10 minutes later.

[1037] Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

Table 6

# of plates	Rxn buffer diluent (ml)	CSPD (ml)	# of plates	Rxn buffer diluent (ml)	CSPD (ml)
10	60	3	31	165	8.25
11	65	3.25	32	170	8.5
12	70	3.5	33	175	8.75
13	75	3.75	34	180	9
14	80	4	35	185	9.25
15	85	4.25	36	190	9.5
16	90	4.5	37	195	9.75
17	95	4.75	38	200	10
18	100	5	39	205	10.25
19	105	5.25	40	210	10.5
20	110	5.5	41	215	10.75
21	115	5.75	42	220	11
22	120	6	43	225	11.25
23	125	6.25	44	230	11.5
24	130	6.5	45	235	11.75
25	135	6.75	46	240	12
26	140	7	47	245	12.25
27	145	7.25	48	250	12.5
28	150	7.5	49	255	12.75
29	155	7.75	50	260	13
30	160	8			

EXAMPLE 26: Assay Identifies Neuronal Activity.

[1038] When cells undergo differentiation and proliferation, a group of genes are activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, the ability of fusion proteins of the invention to activate cells can be assessed.

[1039] Particularly, the following protocol is used to assess neuronal activity in PC12 cell lines. PC12 cells (rat phenochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor). The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells by an albumin fusion protein of the present invention can be assessed.

[1040] The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (-633 to +1)(Sakamoto K et al., Oncogene 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers:

First primer: 5' GCGCTCGAGGGATGACAGCGATAGAACCCCGG-3' (SEQ ID NO:57)

Second primer: 5' GCGAAGCTTCGCGACTCCCCGGATCCGCCTC-3' (SEQ ID NO:58)

[1041] Using the GAS:SEAP/Neo vector produced in Example 24, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes XhoI/HindIII, removing the GAS/SV40 stuffer. Restrict the EGR1 amplified product with these same enzymes. Ligate the vector and the EGR1 promoter.

[1042] To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and allowed to air dry for 2 hr.

[1043] PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 ug/ml streptomycin on a precoated 10 cm tissue culture dish. One to four split is done every three to four days. Cells are removed from the plates by scraping and resuspended with pipetting up and down for more than 15 times.

[1044] Transfect the EGR/SEAP/Neo construct into PC12 using techniques known in the art. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 300 ug/ml G418 for couple of passages.

[1045] To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the

cells once with PBS (Phosphate buffered saline). Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

[1046] The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as $5x10^5$ cells/ml.

[1047] Add 200 ul of the cell suspension to each well of 96-well plate (equivalent to $1x10^5$ cells/well). Add a series of different concentrations of an albumin fusion protein of the invention, 37 degree C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ul of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay may be routinely performed using techniques known in the art and/or as described in Example 25.

EXAMPLE 27: Assay for T-cell Activity

[1048] The following protocol is used to assess T-cell activity by identifying factors, and determining whether an albumin fusion protein of the invention proliferates and/or differentiates T-cells. T-cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 24. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

[1049] Jurkat T-cells are lymphoblastic CD4+ Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml geneticin selected. Resistent colonies are expanded and then tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

[1050] Specifically, the following protocol will yield sufficient cells for 75 wells containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + 10% serum with 1%Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies) with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

[1051] During the incubation period, count cell concentration, spin down the required number of cells (10^7 per transfection), and resuspend in OPTI-MEM to a final concentration of 10^7 cells/ml. Then add 1ml of 1 x 10^7 cells in OPTI-MEM to T25 flask and incubate at 37 degree C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

[1052] The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Geneticin, and 1% Pen-Strep. These cells are treated with varying concentrations of one or more fusion proteins of the present invention.

[1053] On the day of treatment with the fusion protein, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of fusion proteins and the number of different concentrations of fusion proteins being screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

[1054] The well dishes containing Jurkat cells treated with the fusion protein are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 ul samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophene covers) and stored at -20 degree C until SEAP assays are performed according to Example 25. The plates containing the remaining treated cells are placed at 4 degree C and serve as a source of material for repeating the assay on a specific well if desired.

[1055] As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

[1056] The above protocol may be used in the generation of both transient, as well as, stable transfected cells, which would be apparent to those of skill in the art.

EXAMPLE 28: Assay for T-cell Activity

[1057] NF-KB (Nuclear Factor KB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-KB regulates the expression of genes involved in immune cell activation, control of apoptosis (NF- KB appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

[1058] In non-stimulated conditions, NF- KB is retained in the cytoplasm with I-KB (Inhibitor KB). However, upon stimulation, I- KB is phosphorylated and degraded, causing NF- KB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF- KB include IL-2, IL-6, GM-CSF, ICAM-1 and class 1 MHC.

[1059] Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-KB promoter element are used to screen the fusion protein. Activators or inhibitors of NF-KB would be useful in treating, preventing, and/or diagnosing diseases. For example, inhibitors of NF-KB could be used to treat those diseases related to the acute or chronic activation of NF-KB, such as rheumatoid arthritis.

[1060] To construct a vector containing the NF-KB promoter element, a PCR based strategy is employed. The upstream primer contains four

tandem copies of the NF-KB binding site (GGGGACTTTCCC) (SEQ ID NO:59), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:

5':GCGGCCTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGACTTTCCATCCTGCCATCTCAATTAG:3' (SEQ ID NO:60)

[1061] The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site:

5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:55)

[1062] PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI and Hind III and subcloned into BLSK2-. (Stratagene) Sequencing with the T7 and T3 primers confirms the insert contains the following sequence:

5':CTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGACTTTCCATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGC
CCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAG
GCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTT:3' (SEQ
ID NO:61)

[1063] Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF-KB/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

[1064] In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes SalI and NotI, and inserted into a vector containing neomycin resistance. Particularly, the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with SalI and NotI.

[1065] Once NF-KB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 25. Similarly, the method for assaying fusion proteins with these stable Jurkat T-cells is also described in Example 25. As a positive control, exogenous TNF alpha (0.1,1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

EXAMPLE 29: Assay Identifying Myeloid Activity.

[1066] The following protocol is used to assess myeloid activity of an albumin fusion protein of the present invention by determining whether the fusion protein proliferates and/or differentiates myeloid cells. Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 24. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

[1067] To transiently transfect U937 cells with the GAS/SEAP/Neo construct produced in Example 24, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest $2x10^7$ U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

[1068] Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375 uM $Na_2HPO_4.7H_2O$, 1 mM $MgCl_2$, and 675 uM $CaCl_2$. Incubate at 37 degrees C for 45 min.

[1069] Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37 degree C for 36 hr.

[1070] The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

[1071] These cells are tested by harvesting $1x10^8$ cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described growth medium, with a final density of $5x10^5$ cells/ml. Plate 200 ul cells per well in the 96-well plate (or $1x10^5$ cells/well).

[1072] Add different concentrations of the fusion protein. Incubate at 37 degee C for 48 to 72 hr. As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate U937 cells. Over 30 fold induction is typically observed in the positive control wells. SEAP assay the supernatant according to methods known in the art and/or the protocol described in Example 25.

EXAMPLE 30: Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability.

[1073] Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify fusion proteins which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

[1074] The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-4 (Molecular Probes, Inc.; catalog no. F-14202), used here.

[1075] For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in

a CO_2 incubator for 20 hours. The adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank's Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

[1076] A stock solution of 1 mg/ml fluo-4 is made in 10% pluronic acid DMSO. To load the cells with fluo-4, 50 ul of 12 ug/ml fluo-4 is added to each well. The plate is incubated at 37 degrees C in a CO_2 incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 ul of buffer.

[1077] For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to 2-5x10^6 cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml fluo-4 solution in 10% pluronic acid DMSO is added to each ml of cell suspension. The tube is then placed in a 37 degrees C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to 1x10^6 cells/ml, and dispensed into a microplate, 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley Cell Wash with 200 ul, followed by an aspiration step to 100 ul final volume.

[1078] For a non-cell based assay, each well contains a fluorescent molecule, such as fluo-4 . The fusion protein of the invention is added to the well, and a change in fluorescence is detected.

[1079] To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50 ul. Increased emission at 530 nm indicates an extracellular signaling event caused by an albumin fusion protein of the present invention or a molecule induced by an albumin fusion protein of the present invention, which has resulted in an increase in the intracellular Ca^{++} concentration.

EXAMPLE 31: Assay Identifying Tyrosine Kinase Activity.

[1080] The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase (RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

[1081] Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

[1082] Because of the wide range of known factors capable of stimulating tyrosine kinase activity, identifying whether an albumin fusion protein of the present invention or a molecule induced by a fusion protein of the present invention is capable of activating tyrosine kinase signal transduction pathways is of interest. Therefore, the following protocol is designed to identify such molecules capable of activating the tyrosine kinase signal transduction pathways.

[1083] Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford,MA), or calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford,MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

[1084] To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20 minutes treatment with EGF (60ng/ml) or a different concentrations of an albumin fusion protein of the invention, the medium was removed and 100 ml of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na3VO4, 2 mM Na4P2O7 and a cocktail of protease inhibitors (# 1836170) obtained from Boehringer Mannheim (Indianapolis, IN)) is added to each well and the plate is shaken on a rotating shaker for 5 minutes at 4°C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4 degree C at 16,000 x g.

[1085] Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

[1086] Generally, the tyrosine kinase activity of an albumin fusion protein of the invention is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a biotinylated peptide). Biotinylated peptides that can be used for this purpose include

PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

[1087] The tyrosine kinase reaction is set up by adding the following components in order. First, add 10ul of 5uM Biotinylated Peptide, then 10ul ATP/Mg_{2+} (5mM ATP/50mM $MgCl_2$), then 10ul of 5x Assay Buffer (40mM imidazole hydrochloride, pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM $MgCl_2$, 5 mM $MnCl_2$, 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the components gently and preincubate the reaction mix at 30 degree C for 2 min. Initial the reaction by adding 10ul of the control enzyme or the filtered supernatant.

[1088] The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mm EDTA and place the reactions on ice.

[1089] Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37 degree C for 20 min. This allows the streptavidin coated 96 well plate to associate with the biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phosphotyrosine antibody conjugated to horse radish peroxidase(anti-P-Tyr-POD(0.5u/ml)) to each well and incubate at 37 degree C for one hour. Wash the well as above.

[1090] Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

EXAMPLE 32: Assay Identifying Phosphorylation Activity.

[1091] As a potential alternative and/or complement to the assay of protein tyrosine kinase activity described in Example 31, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.

[1092] Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against Erk-1 and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules.) After 3-5 rinses with PBS, the plates are stored at 4 degree C until use.

[1093] A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or varying concentrations of the fusion protein of the invention for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

[1094] After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation by the fusion protein of the present invention or a molecule induced by an albumin fusion protein of the present invention.

EXAMPLE 33: Phosphorylation Assay.

[1095] In order to assay for phosphorylation activity of an albumin fusion protein of the invention, a phosphorylation assay as described in U.S. Patent 5,958,405 (which is herein incorporated by reference) is utilized. Briefly, phosphorylation activity may be measured by phosphorylation of a protein substrate using gamma-labeled ^{32}P-ATP and quantitation of the incorporated radioactivity using a gamma radioisotope counter. The fusion protein of the invention is incubated with the protein substrate, ^{32}P-ATP, and a kinase buffer. The ^{32}P incorporated into the substrate is then separated from free ^{32}P-ATP by electrophoresis, and the incorporated ^{32}P is counted and compared to a negative control. Radioactivity counts above the negative control are indicative of phosphorylation activity of the fusion protein.

EXAMPLE 34: Detection of Phosphorylation Activity (Activation) of an Albumin Fusion Protein of the Invention in the Presence of Polypeptide Ligands.

[1096] Methods known in the art or described herein may be used to determine the phosphorylation activity of an albumin fusion protein of the invention. A preferred method of determining phosphorylation activity is by the use of the tyrosine phosphorylation assay as described in US 5,817,471 (incorporated herein by reference).

EXAMPLE 35: Assay for the Stimulation of Bone Marrow CD34+ Cell Proliferation.

[1097] This assay is based on the ability of human CD34+ to proliferate in the presence of hematopoietic growth factors and evaluates the

ability of fusion proteins of the inventon to stimulate proliferation of CD34+ cells.

[1098] It has been previously shown that most mature precursors will respond to only a single signal. More immature precursors require at least two signals to respond. Therefore, to test the effect of fusion proteins of the invention on hematopoietic activity of a wide range of progenitor cells, the assay contains a given fusion protein of the invention in the presence or absence of hematopoietic growth factors. Isolated cells are cultured for 5 days in the presence of Stem Cell Factor (SCF) in combination with tested sample. SCF alone has a very limited effect on the proliferation of bone marrow (BM) cells, acting in such conditions only as a "survival" factor. However, combined with any factor exhibiting stimulatory effect on these cells (e.g., IL-3), SCF will cause a synergistic effect. Therefore, if the tested fusion protein has a stimulatory effect on hematopoietic progenitors, such activity can be easily detected. Since normal BM cells have a low level of cycling cells, it is likely that any inhibitory effect of a given fusion protein might not be detected. Accordingly, assays for an inhibitory effect on progenitors is preferably tested in cells that are first subjected to *in vitro* stimulation with SCF+IL+3, and then contacted with the compound that is being evaluated for inhibition of such induced proliferation.

[1099] Briefly, CD34+ cells are isolated using methods known in the art. The cells are thawed and resuspended in medium (QBSF 60 serum-free medium with 1% L-glutamine (500ml) Quality Biological, Inc., Gaithersburg, MD Cat# 160-204-101). After several gentle centrifugation steps at 200 x g, cells are allowed to rest for one hour. The cell count is adjusted to 2.5 x 10^5 cells/ml. During this time, 100 µl of sterile water is added to the peripheral wells of a 96-well plate. The cytokines that can be tested with an albumin fusion protein of the invention in this assay is rhSCF (R&D Systems, Minneapolis, MN, Cat# 255-SC) at 50 ng/ml alone and in combination with rhSCF and rhIL-3 (R&D Systems, Minneapolis, MN, Cat# 203-ML) at 30 ng/ml. After one hour, 10 µl of prepared cytokines, varying concentrations of an albumin fusion protein of the invention, and 20 µl of diluted cells are added to the media which is already present in the wells to allow for a final total volume of 100 µl. The plates are then placed in a 37°C/5% CO_2 incubator for five days.

[1100] Eighteen hours before the assay is harvested, 0.5 µCi/well of [3H] Thymidine is added in a 10 µl volume to each well to determine the proliferation rate. The experiment is terminated by harvesting the cells from each 96-well plate to a filtermat using the Tomtec Harvester 96. After harvesting, the filtermats are dried, trimmed and placed into OmniFilter assemblies consisting of one OmniFilter plate and one OmniFilter Tray. 60 µl Microscint is added to each well and the plate sealed with TopSeal-A press-on sealing film A bar code 15 sticker is affixed to the first plate for counting. The sealed plates are then loaded and the level of radioactivity determined via the Packard Top Count and the printed data collected for analysis. The level of radioactivity reflects the amount of cell proliferation.

[1101] The studies described in this example test the activity of a given fusion protein to stimulate bone marrow CD34+ cell proliferation. One skilled in the art could easily modify the exemplified studies to test the activity of fusion porteins and polynucleotides of the invention (e.g., gene therapy) as well as agonists and antagonists thereof. The ability of an albumin fusion protein of the invention to stimulate the proliferation of bone marrow CD34+ cells indicates that the albumin fusion protein and/or polynucleotides corresponding to the fusion protein are useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein.

EXAMPLE 36: Assay for Extracellular Matrix Enhanced Cell Response (EMECR).

[1102] The objective of the Extracellular Matrix Enhanced Cell Response (EMECR) assay is to evaluate the ability of fusion proteins of the invention to act on hematopoietic stem cells in the context of the extracellular matrix (ECM) induced signal.

[1103] Cells respond to the regulatory factors in the context of signal(s) received from the surrounding microenvironment. For example, fibroblasts, and endothelial and epithelial stem cells fail to replicate in the absence of signals from the ECM. Hematopoietic stem cells can undergo self-renewal in the bone marrow, but not in *in vitro* suspension culture. The ability of stem cells to undergo self-renewal *in vitro* is dependent upon their interaction with the stromal cells and the ECM protein fibronectin (fn). Adhesion of cells to fn is mediated by the $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrin receptors, which are expressed by human and mouse hematopoietic stem cells. The factor(s) which integrate with the ECM environment and are responsible for stimulating stem cell self-renewal haven not yet been identified. Discovery of such factors should be of great interest in gene therapy and bone marrow transplant applications

[1104] Briefly, polystyrene, non tissue culture treated, 96-well plates are coated with fn fragment at a coating concentration of 0.2 µg/ cm^2. Mouse bone marrow cells are plated (1,000 cells/well) in 0.2 ml of serum-free medium. Cells cultured in the presence of IL-3 (5 ng/ml) + SCF (50 ng/ml) would serve as the positive control, conditions under which little self-renewal but pronounced differentiation of the stem cells is to be expected. Albumin fusion proteins of the invention are tested with appropriate negative controls in the presence and absence of SCF(5.0 ng/ml), where volume of the administed composition containing the albumin fusion protein of the invention represents 10% of the total assay volume. The plated cells are then allowed to grow by incubating in a low oxygen environment (5% CO_2, 7% O_2, and 88% N_2) tissue culture incubator for 7 days. The number of proliferating cells within the wells is then quantitated by measuring thymidine incorporation into cellular DNA. Verification of the positive hits in the assay will require phenotypic characterization of the cells, which can be accomplished by scaling up of the culture system and using appropriate antibody reagents against cell surface antigens and FACScan.

[1105] If a particular fusion protein of the present invention is found to be a stimulator of hematopoietic progenitors, the fusion protein and polynucleotides corresponding to the fusion protein may be useful for example, in the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein. The fusion protein may also be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

[1106] Additionally, the albumin fusion proteins of the invention and polynucleotides encoding albumin fusion proteins of the invention, may also be employed to inhibit the proliferation and differentiation of hematopoietic cells and therefore may be employed to protect bone marrow stem cells from chemotherapeutic agents during chemotherapy. This antiproliferative effect may allow administration of higher doses of chemotherapeutic agents and, therefore, more effective chemotherapeutic treatment.

[1107] Moreover, fusion proteins of the invention and polynucleotides encoding albumin fusion proteins of the invention may also be useful for the treatment and diagnosis of hematopoietic related disorders such as, anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia, since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia.

EXAMPLE 37: Human Dermal Fibroblast and Aortic Smooth Muscle Cell Proliferation.

[1108] An albumin fusion protein of the invention is added to cultures of normal human dermal fibroblasts (NHDF) and human aortic smooth muscle cells (AoSMC) and two co-assays are performed with each sample. The first assay examines the effect of the fusion protein on the proliferation of normal human dermal fibroblasts (NHDF) or aortic smooth muscle cells (AoSMC). Aberrant growth of fibroblasts or smooth muscle cells is a part of several pathological processes, including fibrosis, and restenosis. The second assay examines IL6 production by both NHDF and SMC. IL6 production is an indication of functional activation. Activated cells will have increased production of a number of cytokines and other factors, which can result in a proinflammatory or immunomodulatory outcome. Assays are run with and without co-TNFa stimulation, in order to check for costimulatory or inhibitory activity.

[1109] Briefly, on day 1, 96-well black plates are set up with 1000 cells/well (NHDF) or 2000 cells/well (AoSMC) in 100 µl culture media. NHDF culture media contains: Clonetics FB basal media, 1mg/ml hFGF, 5mg/ml insulin, 50mg/ml gentamycin, 2%FBS, while AoSMC culture media contains Clonetics SM basal media, 0.5 µg/ml hEGF, 5mg/ml insulin, 1µg/ml hFGF, 50mg/ml gentamycin, 50 µg/ml Amphotericin B, 5%FBS. After incubation at 37°C for at least 4-5 hours culture media is aspirated and replaced with growth arrest media. Growth arrest media for NHDF contains fibroblast basal media, 50mg/ml gentamycin, 2% FBS, while growth arrest media for AoSMC contains SM basal media, 50mg/ml gentamycin, 50µg/ml Amphotericin B, 0.4% FBS. Incubate at 37 °C until day 2.

[1110] On day 2, serial dilutions and templates of an albumin fusion protein of the invention are designed such that they always include media controls and known-protein controls. For both stimulation and inhibition experiments, proteins are diluted in growth arrest media. For inhibition experiments, TNFa is added to a final concentration of 2ng/ml (NHDF) or 5ng/ml (AoSMC). Add 1/3 vol media containing controls or an albumin fusion protein of the invention and incubate at 37 degrees C/5% CO_2 until day 5.

[1111] Transfer 60µl from each well to another labeled 96-well plate, cover with a plate-sealer, and store at 4 degrees C until Day 6 (for IL6 ELISA). To the remaining 100 µl in the cell culture plate, aseptically add Alamar Blue in an amount equal to 10% of the culture volume (10µl). Return plates to incubator for 3 to 4 hours. Then measure fluorescence with excitation at 530nm and emission at 590nm using the CytoFluor. This yields the growth stimulation/inhibition data.

[1112] On day 5, the IL6 ELISA is performed by coating a 96 well plate with 50-100 ul/well of Anti-Human IL6 Monoclonal antibody diluted in PBS, pH 7.4, incubate ON at room temperature.

[1113] On day 6, empty the plates into the sink and blot on paper towels. Prepare Assay Buffer containing PBS with 4% BSA. Block the plates with 200 µl/well of Pierce Super Block blocking buffer in PBS for 1-2 hr and then wash plates with wash buffer (PBS, 0.05% Tween-20). Blot plates on paper towels. Then add 50 µl/well of diluted Anti-Human IL-6 Monoclonal, Biotin-labeled antibody at 0.50 mg/mL. Make dilutions of IL-6 stock in media (30, 10, 3, 1, 0.3, 0 ng/ml). Add duplicate samples to top row of plate. Cover the plates and incubate for 2 hours at RT on shaker.

[1114] Plates are washed with wash buffer and blotted on paper towels. Dilute EU-labeled Streptavidin 1:1000 in Assay buffer, and add 100 µl/well. Cover the plate and incubate 1 h at RT. Plates are again washed with wash buffer and blotted on paper towels.

[1115] Add 100 µl/well of Enhancement Solution. Shake for 5 minutes. Read the plate on the Wallac DELFIA Fluorometer. Readings from triplicate samples in each assay were tabulated and averaged.

[1116] A positive result in this assay suggests AoSMC cell proliferation and that the albumin fusion protein may be involved in dermal fibroblast proliferation and/or smooth muscle cell proliferation. A positive result also suggests many potential uses of the fusion protein and polynucleotides encoding the albumin fusion protein. For example, inflammation and immune responses, wound healing, and angiogenesis, as detailed throughout this specification. Particularly, fusion proteins may be used in wound healing and dermal regeneration, as well as the promotion of vasculogenesis, both of the blood vessels and lymphatics. The growth of vessels can be used in the treatment of, for example, cardiovascular

diseases. Additionally, fusion proteins showing antagonistic activity in this assay may be useful in treating diseases, disorders, and/or conditions which involve angiogenesis by acting as an anti-vascular agent (e.g., anti-angiogenesis). These diseases, disorders, and/or conditions are known in the art and/or are described herein, such as, for example, malignancies, solid tumors, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; atherosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uvietis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis. Moreover, albumin fusion proteins that act as antagonists in this assay may be useful in treating anti-hyperproliferative diseases and/or anti-inflammatory known in the art and/or described herein.

EXAMPLE 38: Cellular Adhesion Molecule (CAM) Expression on Endothelial Cells.

[1117] The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

[1118] Briefly, endothelial cells (e.g., Human Umbilical Vein Endothelial cells (HUVECs)) are grown in a standard 96 well plate to confluence, growth medium is removed from the cells and replaced with 100 µl of 199 Medium (10% fetal bovine serum (FBS)). Samples for testing (containing an albumin fusion protein of the invention) and positive or negative controls are added to the plate in triplicate (in 10 µl volumes). Plates are then incubated at 37°C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 µl of 0.1% paraformaldehyde-PBS(with Ca++ and Mg++) is added to each well. Plates are held at 4°C for 30 min. Fixative is removed from the wells and wells are washed 1X with PBS(+Ca,Mg) + 0.5% BSA and drained. 10 µl of diluted primary antibody is added to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 µg/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed three times with PBS(+Ca,Mg) + 0.5% BSA. 20 µl of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution, referred to herein as the working dilution) are added to each well and incubated at 37°C for 30 min. Wells are washed three times with PBS(+Ca,Mg)+0.5% BSA. Dissolve 1 tablet of p-Nitrophenol Phosphate pNPP per 5 ml of glycine buffer (pH 10.4). 100 µl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphotase in glycine buffer: 1:5,000 (10^{0}) > $10^{-0.5}$ > 10^{-1} > $10^{-1.5}$. 5 µl of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 µl of pNNP reagent is then added to each of the standard wells. The plate is incubated at 37°C for 4h. A volume of 50 µl of 3M NaOH is added to all wells. The plate is read on a plate reader at 405 nm using the background subtraction option on blank wells filled with glycine buffer only. Additionally, the template is set up to indicate the concentration of AP-conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

EXAMPLE 39: Alamar Blue Endothelial Cells Proliferation Assay.

[1119] This assay may be used to quantitatively determine protein mediated inhibition of bFGF-induced proliferation of Bovine Lymphatic Endothelial Cells (LECs), Bovine Aortic Endothelial Cells (BAECs) or Human Microvascular Uterine Myometrial Cells (UTMECs). This assay incorporates a fluorometric growth indicator based on detection of metabolic activity. A standard Alamar Blue Proliferation Assay is prepared in EGM-2MV with 10 ng /ml of bFGF added as a source of endothelial cell stimulation. This assay may be used with a variety of endothelial cells with slight changes in growth medium and cell concentration. Dilutions of protein batches to be tested are diluted as appropriate. Serum-free medium (GIBCO SFM) without bFGF is used as a non-stimulated control and Angiostatin or TSP-1 are included as a known inhibitory controls.

[1120] Briefly, LEC, BAECs or UTMECs are seeded in growth media at a density of 5000 to 2000 cells/well in a 96 well plate and placed at 37 degreesC overnight. After the overnight incubation of the cells, the growth media is removed and replaced with GIBCO EC-SFM. The cells are treated with the appropriate dilutions of an albumin fusion protein of the invention or control protein sample(s) (prepared in SFM) in triplicate wells with additional bFGF to a concentration of 10 ng/ ml. Once the cells have been treated with the samples, the plate(s) is/are placed back in the 37° C incubator for three days. After three days 10 ml of stock alamar blue (Biosource Cat# DAL1100) is added to each well and the plate(s) is/are placed back in the 37°C incubator for four hours. The plate(s) are then read at 530nm excitation and 590nm emission using the CytoFluor fluorescence reader. Direct output is recorded in relative fluorescence units.

[1121] Alamar blue is an oxidation-reduction indicator that both fluoresces and changes color in response to chemical reduction of growth

medium resulting from cell growth. As cells grow in culture, innate metabolic activity results in a chemical reduction of the immediate surrounding environment. Reduction related to growth causes the indicator to change from oxidized (non-fluorescent blue) form to reduced (fluorescent red) form (i.e., stimulated proliferation will produce a stronger signal and inhibited proliferation will produce a weaker signal and the total signal is proportional to the total number of cells as well as their metabolic activity). The background level of activity is observed with the starvation medium alone. This is compared to the output observed from the positive control samples (bFGF in growth medium) and protein dilutions.

EXAMPLE 40: Detection of Inhibition of a Mixed Lymphocyte Reaction.

[1122] This assay can be used to detect and evaluate inhibition of a Mixed Lymphocyte Reaction (MLR) by fusion proteins of the *invention*. Inhibition of a MLR may be due to a direct effect on cell proliferation and viability, modulation of costimulatory molecules on interacting cells, modulation of adhesiveness between lymphocytes and accessory cells, or modulation of cytokine production by accessory cells. Multiple cells may be targeted by the albumin fusion protein that inhibit MLR since the peripheral blood mononuclear fraction used in this assay includes T, B and natural killer lymphocytes, as well as monocytes and dendritic cells.

[1123] Albumin fusion proteins of the invention found to inhibit the MLR may find application in diseases associated with lymphocyte and monocyte activation or proliferation. These include, but are not limited to, diseases such as asthma, arthritis, diabetes, inflammatory skin conditions, psoriasis, eczema, systemic lupus erythematosus, multiple sclerosis, glomerulonephritis, inflammatory bowel disease, crohn's disease, ulcerative colitis, arteriosclerosis, cirrhosis, graft vs. host disease, host vs. graft disease, hepatitis, leukemia and lymphoma.

[1124] Briefly, PBMCs from human donors are purified by density gradient centrifugation using Lymphocyte Separation Medium (LSM®, density 1.0770 g/ml, Organon Teknika Corporation, West Chester, PA). PBMCs from two donors are adjusted to 2×10^6 cells/ml in RPMI-1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS and 2 mM glutamine. PBMCs from a third donor is adjusted to 2×10^5 cells/ml. Fifty microliters of PBMCs from each donor is added to wells of a 96-well round bottom microtiter plate. Dilutions of the fusion protein test material (50 µl) is added in triplicate to microtiter wells. Test samples (of the protein of interest) are added for final dilution of 1:4; rhuIL-2 (R&D Systems, Minneapolis, MN, catalog number 202-IL) is added to a final concentration of 1 µg/ml; anti-CD4 mAb (R&D Systems, clone 34930.11, catalog number MAB379) is added to a final concentration of 10 µg/ml. Cells are cultured for 7-8 days at 37°C in 5% CO_2, and 1 µC of [^{3}H] thymidine is added to wells for the last 16 hrs of culture. Cells are harvested and thymidine incorporation determined using a Packard TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

[1125] Samples of the fusion protein of interest are screened in separate experiments and compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes and the positive control treatment, IL-2 (either as recombinant material or supernatant), which enhances proliferation of lymphocytes.

EXAMPLE 41: Assays for Protease Activity.

[1126] The following assay may be used to assess protease activity of an albumin fusion protein of the invention.

[1127] Gelatin and casein zymography are performed essentially as described (Heussen et al., *Anal. Biochem.*, 102:196-202 (1980); Wilson et al., *Journal of Urology*, 149:653-658 (1993)). Samples are run on 10% polyacrylamide/0.1% SDS gels containing 1% gelatin or casein, soaked in 2.5% triton at room temperature for 1 hour, and in 0.1M glycine, pH 8.3 at 37°C 5 to 16 hours. After staining in amido black areas of proteolysis appear as clear areas against the blue-black background. Trypsin (Sigma T8642) is used as a positive control.

[1128] Protease activity is also determined by monitoring the cleavage of n-a-benzoyl-L-arginine ethyl ester (BAEE) (Sigma B-4500. Reactions are set up in (25mMNaPO$_4$,1mM EDTA, and 1mM BAEE), pH 7.5. Samples are added and the change in absorbance at 260nm is monitored on the Beckman DU-6 spectrophotometer in the time-drive mode. Trypsin is used as a positive control.

[1129] Additional assays based upon the release of acid-soluble peptides from casein or hemoglobin measured as absorbance at 280 nm or colorimetrically using the Folin method are performed as described in Bergmeyer, et al., *Methods of Enzymatic Analysis*, 5 (1984). Other assays involve the solubilization of chromogenic substrates (Ward, *Applied Science*, 251-317 (1983)).

EXAMPLE 42: Identifying Serine Protease Substrate Specificity.

[1130] Methods known in the art or described herein may be used to determine the substrate specificity of the albumin fusion proteins of the present invention having serine protease activity. A preferred method of determining substrate specificity is by the use of positional scanning synthetic combinatorial libraries as described in GB 2 324 529 (incorporated herein in its entirety).

EXAMPLE 43: Ligand Binding Assays.

[1131] The following assay may be used to assess ligand binding activity of an albumin fusion protein of the invention.

[1132] Ligand binding assays provide a direct method for ascertaining receptor pharmacology and are adaptable to a high throughput format. The purified ligand for an albumin fusion protein of the invention is radiolabeled to high specific activity (50-2000 Ci/mmol) for binding studies. A determination is then made that the process of radiolabeling does not diminish the activity of the ligand towards the fusion protein. Assay conditions for buffers, ions, pH and other modulators such as nucleotides are optimized to establish a workable signal to noise ratio for both membrane and whole cell polypeptide sources. For these assays, specific polypeptide binding is defined as total associated radioactivity minus the radioactivity

measured in the presence of an excess of unlabeled competing ligand. Where possible, more than one competing ligand is used to define residual nonspecific binding.

EXAMPLE 44: Functional Assay in Xenopus Oocytes.

[1133] Capped RNA transcripts from linearized plasmid templates encoding an albumin fusion protein of the invention is synthesized in vitro with RNA polymerases in accordance with standard procedures. In vitro transcripts are suspended in water at a final concentration of 0.2 mg/ml. Ovarian lobes are removed from adult female toads, Stage V defolliculated oocytes are obtained, and RNA transcripts (10 ng/oocyte) are injected in a 50 nl bolus using a microinjection apparatus. Two electrode voltage clamps are used to measure the currents from individual *Xenopus oocytes* in response fusion protein and polypeptide agonist exposure. Recordings are made in Ca2+ free Barth's medium at room temperature. The Xenopus system can be used to screen known ligands and tissue/cell extracts for activating ligands.

EXAMPLE 45: Microphysiometric Assays.

[1134] Activation of a wide variety of secondary messenger systems results in extrusion of small amounts of acid from a cell. The acid formed is largely as a result of the increased metabolic activity required to fuel the intracellular signaling process. The pH changes in the media surrounding the cell are very small but are detectable by the CYTOSENSOR microphysiometer (Molecular Devices Ltd., Menlo Park, Calif.). The CYTOSENSOR is thus capable of detecting the ability of an albumin fusion protein of the invention to activate secondary messengers that are coupled to an energy utilizing intracellular signaling pathway.

EXAMPLE 46: Extract/Cell Supernatant Screening.

[1135] A large number of mammalian receptors exist for which there remains, as yet, no cognate activating ligand (agonist). Thus, active ligands for these receptors may not be included within the ligands banks as identified to date. Accordingly, the albumin fusion proteins of the invention can also be functionally screened (using calcium, cAMP, microphysiometer, oocyte electrophysiology, etc., functional screens) against tissue extracts to identify natural ligands for the Therapeutic protein portion and/or albumin protein portion of an albumin fusion protein of the invention. Extracts that produce positive functional responses can be sequentially subfractionated until an activating ligand is isolated and identified.

EXAMPLE 47: ATP-binding assay.

[1136] The following assay may be used to assess ATP-binding activity of fusion proteins of the invention.

[1137] ATP-binding activity of an albumin fusion protein of the invention may be detected using the ATP-binding assay described in U.S. Patent 5,858,719, which is herein incorporated by reference in its entirety. Briefly, ATP-binding to an albumin fusion protein of the invention is measured via photoaffinity labeling with 8-azido-ATP in a competition assay. Reaction mixtures containing 1 mg/ml of ABC transport protein are incubated with varying concentrations of ATP, or the non-hydrolyzable ATP analog adenyl-5'-imidodiphosphate for 10 minutes at 4°C. A mixture of 8-azido-ATP (Sigma Chem. Corp., St. Louis, MO.) plus 8-azido-ATP (^{32}P-ATP) (5 mCi/μmol, ICN, Irvine CA.) is added to a final concentration of 100 μM and 0.5 ml aliquots are placed in the wells of a porcelain spot plate on ice. The plate is irradiated using a short wave 254 nm UV lamp at a distance of 2.5 cm from the plate for two one-minute intervals with a one-minute cooling interval in between. The reaction is stopped by addition of dithiothreitol to a final concentration of 2mM. The incubations are subjected to SDS-PAGE electrophoresis, dried, and autoradiographed. Protein bands corresponding to the albumin fusion proteins of the invention are excised, and the radioactivity quantified. A decrease in radioactivity with increasing ATP or adenly-5'-imidodiphosphate provides a measure of ATP affinity to the fusion protein.

EXAMPLE 48: Identification Of Signal Transduction Proteins That Interact With An albumin fusion protein Of The Present Invention.

[1138] Albumin fusion proteins of the invention may serve as research tools for the identification, characterization and purification of signal transduction pathway proteins or receptor proteins. Briefly, a labeled fusion protein of the invention is useful as a reagent for the purification of molecules with which it interacts. In one embodiment of affinity purification, an albumin fusion protein of the invention is covalently coupled to a chromatography column. Cell-free extract derived from putative target cells, such as carcinoma tissues, is passed over the column, and molecules with appropriate affinity bind to the albumin fusion protein. The protein complex is recovered from the column, dissociated, and the recovered molecule subjected to N-terminal protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

EXAMPLE 49: IL-6 Bioassay.

[1139] A variety of assays are known in the art for testing the proliferative effects of an albumin fusion protein of the invention. For example, one such assay is the IL-6 Bioassay as described by Mosmann *et al.* (*Proc. Natl. Acad. Sci., U.S.A.*, 95:3251-56 (1998), which is herein incorporated by reference). After 68 hrs. at 37°C, the number of viable cells is measured by adding the tetrazolium salt thiazolyl blue (MTT) and incubating for a further 4 hrs. at 37°C. B9 cells are lysed by SDS and optical density is measured at 570 nm. Controls containing IL-6 (positive) and no cytokine (negative) are Briefly, IL-6 dependent B9 murine cells are washed three times in IL-6 free medium and plated at a concentration of 5,000 cells per well in 50 μl, and 50 μl of fusion protein of the invention is added. utilized. Enhanced proliferation in the test sample(s) (containing an albumin fusion protein of the invention) relative to the negative control is indicative of proliferative effects mediated by the fusion protein.

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EXAMPLE 50: Support of Chicken Embryo Neuron Survival.

[1140] To test whether sympathetic neuronal cell viability is supported by an albumin fusion protein of the invention, the chicken embryo neuronal survival assay of Senaldi *et al* may be utilized (*Proc. Natl. Acad. Sci., U.S.A., 96*:11458-63 (1998), which is herein incorporated by reference). Briefly, motor and sympathetic neurons are isolated from chicken embryos, resuspended in L15 medium (with 10% FCS, glucose, sodium selenite, progesterone, conalbumin, putrescine, and insulin; Life Technologies, Rockville, MD.) and Dulbecco's modified Eagles medium [with 10% FCS, glutamine, penicillin, and 25 mM Hepes buffer (pH 7.2); Life Technologies, Rockville, MD.], respectively, and incubated at 37°C in 5% CO_2 in the presence of different concentrations of the purified fusion protein of the invention, as well as a negative control lacking any cytokine. After 3 days, neuron survival is determined by evaluation of cellular morphology, and through the use of the colorimetric assay of Mosmann (Mosmann, T., *J. Immunol. Methods, 65*:55-63 (1983)). Enhanced neuronal cell viability as compared to the controls lacking cytokine is indicative of the ability of the albumin fusion protein to enhance the survival of neuronal cells.

EXAMPLE 51: Assay for Phosphatase Activity.

[1141] The following assay may be used to assess serine/threonine phosphatase (PTPase) activity of an albumin fusion protein of the invention.

[1142] In order to assay for serine/threonine phosphatase (PTPase) activity, assays can be utilized which are widely known to those skilled in the art. For example, the serine/threonine phosphatase (PSPase) activity of an albumin fusion protein of the invention may be measured using a PSPase assay kit from New England Biolabs, Inc. Myelin basic protein (MyBP), a substrate for PSPase, is phosphorylated on serine and threonine residues with cAMP-dependent Protein Kinase in the presence of [^{32}P]ATP. Protein serine/threonine phosphatase activity is then determined by measuring the release of inorganic phosphate from 32P-labeled MyBP.

EXAMPLE 52: Interaction of Serine/Threonine Phosphatases with other Proteins.

[1143] Fusion proteins of the invention having serine/threonine phosphatase activity (e.g., as determined in Example 51) are useful, for example, as research tools for the identification, characterization and purification of additional interacting proteins or receptor proteins, or other signal transduction pathway proteins. Briefly, a labeled fusion protein of the invention is useful as a reagent for the purification of molecules with which it interacts. In one embodiment of affinity purification, an albumin fusion protein of the invention is covalently coupled to a chromatography column. Cell-free extract derived from putative target cells, such as neural or liver cells, is passed over the column, and molecules with appropriate affinity bind to the fusion protein. The fusion protein -complex is recovered from the column, dissociated, and the recovered molecule subjected to N-terminal protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

EXAMPLE 53: Assaying for Heparanase Activity.

[1144] There a numerous assays known in the art that may be employed to assay for heparanase activity of an albumin fusion protein of the invention. In one example, heparanase activity of an albumin fusion protein of the invention, is assayed as described by Vlodavsky et al., (Vlodavsky et al., Nat. Med., 5:793-802 (1999)). Briefly, cell lysates, conditioned media, intact cells (1×10^5 cells per 35-mm dish), cell culture supernatant, or purified fusion protein are incubated for 18 hrs at 37°C, pH 6.2-6.6, with ^{35}S-labeled ECM or soluble ECM derived peak 1 proteoglycans. The incubation medium is centrifuged and the supernatant is analyzed by gel filtration on a Sepharose CL-6B column (0.9 x 30 cm). Fractions are eluted with PBS and their radioactivity is measured. Degradation fragments of heparan sulfate side chains are eluted from Sepharose 6B at $0.5 < K_{av} < 0.8$ (peak II). Each experiment is done at least three times. Degradation fragments corresponding to "peak II," as described by Vlodavsky et al., is indicative of the activity of an albumin fusion protein of the invention in cleaving heparan sulfate.

EXAMPLE 54: Immobilization of Biomolecules.

[1145] This example provides a method for the stabilization of an albumin fusion protein of the invention in non-host cell lipid bilayer constructs (see, e.g., Bieri et al., Nature Biotech 17:1105-1108 (1999), hereby incorporated by reference in its entirety herein) which can be adapted for the study of fusion proteins of the invention in the various functional assays described above. Briefly, carbohydrate-specific chemistry for biotinylation is used to confine a biotin tag to an albumin fusion protein of the invention, thus allowing uniform orientation upon immobilization. A 50uM solution of an albumin fusion protein of the invention in washed membranes is incubated with 20 mM NaIO4 and 1.5 mg/ml (4mM) BACH or 2 mg/ml (7.5mM) biotin-hydrazide for 1 hr at room temperature (reaction volume, 150ul). Then the sample is dialyzed (Pierce Slidealizer Cassett, 10 kDa cutoff; Pierce Chemical Co., Rockford IL) at 4C first for 5 h, exchanging the buffer after each hour, and finally for 12 h against 500 ml buffer R (0.15 M NaCl, 1 mM MgCl2, 10 mM sodium phosphate, pH7). Just before addition into a cuvette, the sample is diluted 1:5 in buffer ROG50 (Buffer R supplemented with 50 mM octylglucoside).

EXAMPLE 55: Assays for Metalloproteinase Activity.

[1146] Metalloproteinases are peptide hydrolases which use metal ions, such as Zn^{2+}, as the catalytic mechanism. Metalloproteinase activity of an albumin fusion protein of the present invention can be assayed according to methods known in the art. The following exemplary methods are provided:

Proteolysis of alpha-2-macroglobulin

[1147] To confirm protease activity, a purified fusion protein of the invention is mixed with the substrate alpha-2-macroglobulin (0.2 unit/ml;

Boehringer Mannheim, Germany) in 1x assay buffer (50 mM HEPES, pH 7.5, 0.2 M NaCl, 10 mM $CaCl_2$, 25 μM $ZnCl_2$ and 0.05% Brij-35) and incubated at 37°C for 1-5 days. Trypsin is used as positive control. Negative controls contain only alpha-2-macroglobulin in assay buffer. The samples are collected and boiled in SDS-PAGE sample buffer containing 5% 2-mercaptoethanol for 5-min, then loaded onto 8% SDS-polyacrylamide gel. After electrophoresis the proteins are visualized by silver staining. Proteolysis is evident by the appearance of lower molecular weight bands as compared to the negative control.

Inhibition of alpha-2-macroglobulin proteolysis by inhibitors of metalloproteinases

[1148] Known metalloproteinase inhibitors (metal chelators (EDTA, EGTA, AND $HgCl_2$), peptide metalloproteinase inhibitors (TIMP-1 and TIMP-2), and commercial small molecule MMP inhibitors) may also be used to characterize the proteolytic activity of an albumin fusion protein of the invention. Three synthetic MMP inhibitors that may be used are: MMP inhibitor I, [IC_{50} = 1.0 μM against MMP-1 and MMP-8; IC_{50} = 30 μM against MMP-9; IC_{50} = 150 μM against MMP-3]; MMP-3 (stromelysin-1) inhibitor I [IC_{50} = 5 μM against MMP-3], and MMP-3 inhibitor II [K_i = 130 nM against MMP-3]; inhibitors available through Calbiochem, catalog # 444250, 444218, and 444225, respectively). Briefly, different concentrations of the small molecule MMP inhibitors are mixed with a purified fusion protein of the invention (50μg/ml) in 22.9 μl of 1x HEPES buffer (50 mM HEPES, pH 7.5, 0.2 M NaCl, 10 mM $CaCl_2$, 25 μM $ZnCl_2$ and 0.05%Brij-35) and incubated at room temperature (24 °C) for 2-hr, then 7.1 μl of substrate alpha-2-macroglobulin (0.2 unit/ml) is added and incubated at 37°C for 20-hr. The reactions are stopped by adding 4x sample buffer and boiled immediately for 5 minutes. After SDS-PAGE, the protein bands are visualized by silver stain.

Synthetic Fluorogenic Peptide Substrates Cleavage Assay

[1149] The substrate specificity for fusion proteins of the invention with demonstrated metalloproteinase activity may be determined using techniques known in the art, such as using synthetic fluorogenic peptide substrates (purchased from BACHEM Bioscience Inc). Test substrates include, M-1985, M-2225, M-2105, M-2110, and M-2255. The first four are MMP substrates and the last one is a substrate of tumor necrosis factor-α (TNF-α) converting enzyme (TACE). These substrates are preferably prepared in 1:1 dimethyl sulfoxide (DMSO) and water. The stock solutions are 50-500 μM. Fluorescent assays are performed by using a Perkin Elmer LS 50B luminescence spectrometer equipped with a constant temperature water bath. The excitation λ is 328 nm and the emission λ is 393 nm. Briefly, the assay is carried out by incubating 176 μl 1x HEPES buffer (0.2 M NaCl, 10 mM $CaCl_2$, 0.05% Brij-35 and 50 mM HEPES, pH 7.5) with 4 μl of substrate solution (50 μM) at 25 °C for 15 minutes, and then adding 20 μl of a purified fusion protein of the invention into the assay cuvett. The final concentration of substrate is 1 μM. Initial hydrolysis rates are monitored for 30-min.

EXAMPLE 36: Occurrence of Diabetes in NOD Mice.

[1150] Female NOD (non-obese diabetic) mice are characterized by displaying IDDM with a course which is similar to that found in humans, although the disease is more pronounced in female than male NOD mice. Hereinafter, unless otherwise stated, the term "NOD mouse" refers to a female NOD mouse. NOD mice have a progressive destruction of beta cells which is caused by a chronic autoimmune disease. Thus, NOD mice begin life with euglycemia, or normal blood glucose levels. By about 15 to 16 weeks of age, however, NOD mice start becoming hyperglycemic, indicating the destruction of the majority of their pancreatic beta cells and the corresponding inability of the pancreas to produce sufficient insulin. Thus, both the cause and the progression of the disease are similar to human IDDM patients.

[1151] *In vivo* assays of efficacy of the immunization regimens can be assessed in female NOD/LtJ mice (commercially available from The Jackson Laboratory, Bar Harbor, Me.). In the literature, it's reported that 80% of female mice develop diabetes by 24 weeks of age and onset of insulitis begins between 6-8 weeks age. NOD mice are inbred and highly responsive to a variety of immunoregulatory strategies. Adult NOD mice (6-8 weeks of age) have an average mass of 20-25 g.

[1152] These mice can be either untreated (control), treated with the therapeutics of the subject invention (e.g., albumin fusion proteins of the invention and fragments and variants thereof), alone or in combination with other therapeutic compounds stated above. The effect of these various treatments on the progression of diabetes can be measured as follows:

[1153] At 14 weeks of age, the female NOD mice can be phenotyped according to glucose tolerance. Glucose tolerance can be measured with the intraperitoneal glucose tolerance test (IPGTT). Briefly, blood is drawn from the paraorbital plexus at 0 minutes and 60 minutes after the intraperitoneal injection of glucose (1 g/kg body weight). Normal tolerance is defined as plasma glucose at 0 minutes of less than 144 mg %, or at 60 minutes of less than 160 mg %. Blood glucose levels are determined with a Glucometer Elite apparatus.

[1154] Based upon this phenotypic analysis, animals can be allocated to the different experimental groups. In particular, animals with more elevated blood glucose levels can be assigned to the impaired glucose tolerance group. The mice can be fed *ad libitum* and can be supplied with acidified water (pH 2.3).

[1155] The glucose tolerant and intolerant mice can be further subdivided into control, albumin fusion proteins of the subject invention, and albumin fusion proteins/therapeutic compounds combination groups. Mice in the control group can receive an interperitoneal injection of vehicle daily, six times per week. Mice in the albumin fusion group can receive an interperitoneal injection of the therapeutics of the subject invention (e.g., albumin fusion proteins of the invention and fragments and variants thereof) in vehicle daily, six times per week. Mice in the albumin fusion

proteins/therapeutic compounds combination group can receive both albumin fusion proteins and combinations of therapeutic compounds as described above.

[1156] The level of urine glucose in the NOD mice can be determined on a bi-weekly basis using Labstix (Bayer Diagnostics, Hampshire, England). Weight and fluid intake can also be determined on a bi-weekly basis. The onset of diabetes is defined after the appearance of glucosuria on two consecutive determinations. After 10 weeks of treatment, an additional IPGTT can be performed and animals can be sacrificed the following day.

[1157] Over the 10 week course of treatment, control animals in both the glucose tolerant and glucose intolerant groups develop diabetes at a rate of 60% and 86%, respectively (see US patent No. 5,866,546, Gross et al.). Thus, high rates of diabetes occur even in NOD mice which are initially glucose tolerant if no intervention is made.

[1158] Results can be confirmed by the measurement of blood glucose levels in NOD mice, before and after treatment. Blood glucose levels are measured as described above in both glucose tolerant and intolerant mice in all groups described.

[1159] In an alternative embodiment, the therapeutics of the subject invention (e.g., specific fusions disclosed as SEQ ID NO:Y and fragments and variants thereof) can be quantified using spectrometric analysis and appropriate protein quantities can be resuspended prior to injection in 50 .mu.l-phosphate buffered saline (PBS) per dose. Two injections, one week apart, can be administered subcutaneously under the dorsal skin of each mouse. Monitoring can be performed on two separate occasions prior to immunization and can be performed weekly throughout the treatment and continued thereafter. Urine can be tested for glucose every week (Keto-Diastix.RTM.; Miles Inc., Kankakee, Ill.) and glycosuric mice can be checked for serum glucose (ExacTech.RTM., MediSense, Inc., Waltham, Mass.). Diabetes is diagnosed when fasting glycemia is greater than 2.5g/L.

EXAMPLE 57: Histological Examination of NOD Mice.

[1160] Histological examination of tissue samples from NOD mice can demonstrate the ability of the compositions of the present invention, and/or a combination of the compositions of the present invention with other therapeutic agents for diabetes, to increase the relative concentration of beta cells in the pancreas. The experimental method is as follows:

[1161] The mice from Example 56 can be sacrificed at the end of the treatment period and tissue samples can be taken from the pancreas. The samples can be fixed in 10% formalin in 0.9% saline and embedded in wax. Two sets of 5 serial 5 .mu.m sections can be cut for immunolabelling at a cutting interval of 150 .mu.m. Sections can be immunolabelled for insulin (guinea pig anti-insulin antisera dilution 1:1000, ICN Thames U.K.) and glucagon (rabbit anti-pancreatic glucagon antisera dilution 1:2000) and detected with peroxidase conjugated anti-guinea pig (Dako, High Wycombe, U.K.) or peroxidase conjugated anti-rabbit antisera (dilution 1:50, Dako).

[1162] The composition of the present invention may or may not have as strong an effect on the visible mass of beta cells as it does on the clinical manifestations of diabetes in glucose tolerant and glucose intolerant animals.

EXAMPLE 58: In vivo Mouse Model of NIDDM.

[1163] Male C57BL/6J mice from Jackson Laboratory (Bar Harbor, ME) can be obtained at 3 weeks of age and fed on conventional chow or diets enriched in either fat (35.5% wt/wt; Bioserv.Frenchtown, NJ) or fructose (60% wt/wt; Harlan Teklad, Madison, WI). The regular chow is composed of 4.5% wt/wt fat, 23% wt/wt protein, 31.9% wt/wt starch, 3.7% wt/wt fructose, and 5.3% wt/wt fiber. The high-fat (lard) diet is composed of 35.5% wt/wt fat, 20% wt/wt protein, 36.4% wt/wt starch, 0.0% wt/wt fructose, and 0.1% wt/wt fiber. The high-fructose diet is composed of 5% wt/wt fat, 20% wt/wt protein, 0.0% wt/wt starch, 60% wt/wt fructose, and 9.4% wt/wt fiber. The mice may be housed no more than five per cage at 22° +/- 3°C temperature- and 50% +/- 20% humidity-controlled room with a 12-hour light (6 am to 6 pm)/dark cycle (Luo et al., 1998, Metabolism 47(6): 663-8, "Nongenetic mouse models of non-insulin-dependent diabetes mellitus"; Larsen et al., Diabetes 50(11): 2530-9 (2001), "Systemic administration of the long-acting GLP-1 derivative NN2211 induces lasting and reversible weight loss in both normal and obese rats"). After exposure to the respective diets for 3 weeks, mice can be injected intraperitoneally with either streptozotocin, "STZ" (Sigma, St. Louis, MO), at 100 mg/kg body weight or vehicle (0.05 mol/L citric acid, pH 4.5) and kept on the same diet for the next 4 weeks. Under nonfasting conditions, blood is obtained 1, 2, and 4 weeks post-STZ by nipping the distal part of the tail. Samples are used to measure nonfasting plasma glucose and insulin concentrations. Body weight and food intake are recorded weekly.

[1164] To directly determine the effect of the high-fat diet on the ability of insulin to stimulate glucose disposal, the experiments can be initiated on three groups of mice, fat-fed, chow-fed injected with vehicle, and fat-fed injected with STZ at the end of the 7-week period described above. Mice can be fasted for 4 hours before the experiments. In the first series of experiments, mice can be anesthetized with methoxyflurane (Pitman-Moor, Mundelein, IL) inhalation. Regular insulin (Sigma) can be injected intravenously ([IV] 0.1 U/kg body weight) through a tail vein, and blood can be collected 3, 6, 9, 12, and 15 minutes after the injection from a different tail vein. Plasma glucose concentrations can be determined on these samples, and the half-life (t½) of glucose disappearance from plasma can be calculated using WinNonlin (Scientific Consulting, Apex, NC), a pharmacokinetics/pharmacodynamics software program.

[1165] In the second series of experiments, mice can be anesthetized with intraperitoneal sodium pentobarbital (Sigma). The abdominal cavity is opened, and the main abdominal vein is exposed and catheterized with a 24-gauge IV catheter (Johnson-Johnson Medical, Arlington, TX). The

catheter is secured to muscle tissue adjacent to the abdominal vein, cut on the bottom of the syringe connection, and hooked to a prefilled PE50 plastic tube, which in turn is connected to a syringe with infusion solution. The abdominal cavity is then sutured closed. With this approach, there would be no blockage of backflow of the blood from the lower part of the body. Mice can be infused continuously with glucose (24.1 mg/kg/min) and insulin (10 mU/kg/min) at an infusion volume of 10 μL/min. Retro-orbital blood samples (70 μL each) can be taken 90, 105, 120, and 135 minutes after the start of infusion for measurement of plasma glucose and insulin concentrations. The mean of these four samples is used to estimate steady-state plasma glucose (SSPG) and insulin (SSPI) concentrations for each animal.

[1166] *Finally, experiments to evaluate the ability of the albumin fusion proteins, the therapeutic compositions of the instant application, either* alone or in combination with any one or more of the therapeutic drugs listed for the treatment of diabetes mellitus, to decrease plasma glucose can be performed in the following two groups of "NIDDM" mice models that are STZ-injected: (1) fat-fed C57BL/6J, and (2) fructose-fed C57BL/6J. Plasma glucose concentrations of the mice for these studies may range from 255 to 555 mg/dL. Mice are randomly assigned to treatment with either vehicle, albumin fusion therapeutics of the present invention either alone or in combination with any one or more of the therapeutic drugs listed for the treatment of diabetes mellitus. A total of three doses can be administered. Tail vein blood samples can be taken for measurement of the plasma glucose concentration before the first dose and 3 hours after the final dose.

[1167] Plasma glucose concentrations can be determined using the Glucose Diagnostic Kit from Sigma (Sigma No. 315), an enzyme colorimetric assay. Plasma insulin levels can be determined using the Rat Insulin RIA Kit from Linco Research (#RI-13K; St. Charles, MO).

EXAMPLE 33: In vitro H4IIe –SEAP Reporter Assay Establishes Involvement in Insulin Action.

The Various H4IIe Reporters

[1168] *H4IIe/rMEP-SEAP*: The malic enzyme promoter isolated from rat (rMEP) contains a PPAR-gamma element which is in the insulin pathway. This reporter construct is stably transfected into the liver H4IIe cell-line.

[1169] *H4IIe/SREBP-SEAP*: The sterol regulatory element binding protein (SREBP-1c) is a transcription factor which acts on the promoters of a number of insulin-responsive genes, for example, fatty acid synthetase (FAS), and which regulates expression of key genes in fatty acid metabolism in fibroblasts, adipocytes, and hepatocytes. SREBP-1c, also known as the adipocyte determination and differentiation factor 1 (ADD-1), is considered as the primary mediator of insulin effects on gene expression in adipose cells. It's activity is modulated by the levels of insulin, sterols, and glucose. This reporter construct is stably transfected into the liver H4IIe cell-line.

[1170] *H4IIe/FAS-SEAP*: The fatty acid synthetase reporter constructs contain a minimal SREBP-responsive FAS promoter. This reporter construct is stably transfected into the liver H4IIe cell-line.

[1171] *H4IIe/PEPCK-SEAP*: The phosphoenolpyruvate carboxykinase (PEPCK) promoter is the primary site of hormonal regulation of PEPCK gene transcription modulating PEPCK activity. PEPCK catalyzes a committed and rate-limiting step in hepatic gluconeogenesis and must therefore be carefully controlled to maintain blood glucose levels within normal limits. This reporter construct is stably transfected into the liver H4IIe cell-line.

[1172] These reporter constructs can also be stably transfected into 3T3-L1 fibroblasts and L6 myoblasts. These stable cell-lines are then differentiated into 3T3-L1 adipocytes and L6 myotubes as previously described in Example 13. The differentiated cell-lines can then be used in the SEAP assay described below.

Growth and Assay Medium

[1173] The growth medium comprises 10% Fetal Bovine Serum (FBS), 10% Calf Serum, 1% NEAA, 1x penicillin/streptomycin, and 0.75 mg/mL G418 (for H4IIe/rFAS-SEAP and H4IIe/SREBP-SEAP) or 0.50 mg/mL G418 (for H4IIe/rMEP-SEAP). For H4IIe/PEPCK-SEAP, the growth medium consists of 10% FBS, 1% penicillin/streptomycin, 15 mM HEPES buffered saline, and 0.50 mg/mL G418.

[1174] The assay medium consists of low glucose DMEM medium (Life Technologies), 1% NEAA, 1x penicillin/streptomycin for the H4IIe/rFAS-SEAP, H4IIe/SREBP-SEAP, H4IIe/rMEP-SEAP reporters. The assay medium for H4IIe/PEPCK-SEAP reporter consists of 0.1% FBS, 1% penicillin/streptomycin, and 15 mM HEPES buffered saline.

Method

[1175] The 96-well plates are seeded at 75,000 cells/well in 100 μL/well of growth medium until cells in log growth phase become adherent. Cells are starved for 48 hours by replacing growth medium with assay medium, 200 μL/well. (For H4IIe/PEPCK-SEAP cells, assay medium containing 0.5 μM dexamethasone is added at 100 μL/well and incubated for approximately 20 hours). The assay medium is replaced thereafter with 100 μL/well of fresh assay medium, and a 50 μL aliquot of cell supernatant obtained from transfected cell-lines expressing the therapeutics of the subject invention (e.g., albumin fusion proteins of the invention and fragments and variants thereof) is added to the well. Supernatants from empty vector transfected cell-lines are used as negative control. Addition of 10 nM and/or 100 nM insulin to the wells is used as positive control. After 48 hours of incubation, the conditioned media are harvested and SEAP activity measured (Phospha-Light System protocol, Tropix #BP2500). Briefly, samples are diluted 1:4 in dilution buffer and incubated at 65 °C for 30 minutes to inactivate the endogenous non-placental form of SEAP. An aliquot of 50 μL of the diluted samples is mixed with 50 μL of SEAP Assay Buffer which contains a mixture of inhibitors active against the non-

placental SEAP isoenzymes and is incubated for another 5 minutes. An aliquot of 50 µL of CSPD chemiluminescent substrate which is diluted 1:20 in Emerald luminescence enhancer is added to the mixture and incubated for 15-20 minutes. Plates are read in a Dynex plate luminometer.

EXAMPLE 60: Transgenic Animals.

[1176] The albumin fusion proteins of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, e.g., baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express fusion proteins of the invention in humans, as part of a gene therapy protocol.

[1177] Any technique known in the art may be used to introduce the polynucleotides encoding the albumin fusion proteins of the invention into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e.g., Ulmer et al., Science 259:1745 (1993); introducing nucleic acid constructs into embryonic pleuripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., Cell 57:717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

[1178] Any technique known in the art may be used to produce transgenic clones containing polynucleotides encoding albumin fusion proteins of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

[1179] The present invention provides for transgenic animals that carry the polynucleotides encoding the albumin fusion proteins of the invention in all their cells, as well as animals which carry these polynucleotides in some, but not all their cells, i.e., mosaic animals or chimeric. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, e.g., head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide encoding the fusion protein of the invention be integrated into the chromosomal site of the endogenous gene corresponding to the Therapeutic protein portion or albumin portion of the fusion protein of the invention, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

[1180] Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the polynucleotide encoding the fusion protien of the invention has taken place. The level of mRNA expression of the polynucleotide encoding the fusion protein of the invention in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of fusion protein-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the fusion protein.

[1181] Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene (i.e., polynucleotide encoding an albumin fusion protein of the invention) on a distinct background that is appropriate for an experimental model of interest.

Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of fusion proteins of the invention and the Therapeutic protein and/or albumin component of the fusion protein of the invention, studying conditions and/or disorders associated with aberrant expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

EXAMPLE 61: Method of Treatment Using Gene Therapy-Ex Vivo.

[1182] One method of gene therapy transplants fibroblasts, which are capable of expressing an albumin fusion protein of the present invention, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

[1183] At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

[1184] pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

[1185] Polynucleotides encoding an albumin fusion protein of the invention can be generated using techniques known in the art amplified using PCR primers which correspond to the 5' and 3' end sequences and optionally having appropriate restriction sites and initiation/stop codons, if necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

[1186] The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

[1187] Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether the albumin fusion protein is produced.

[1188] The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

EXAMPLE 62: Method of Treatment Using Gene Therapy - In Vivo.

[1189] Another aspect of the present invention is using *in vivo* gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences encoding an albumin fusion protein of the invention into an animal. Polynucleotides encoding albumin fusion proteins of the present invention may be operatively linked to (i.e., associated with) a promoter or any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata et al., Cardiovasc. Res. 35(3):470-479 (1997); Chao et al., Pharmacol. Res. 35(6):517-522 (1997); Wolff, Neuromuscul. Disord. 7(5):314-318 (1997); Schwartz et al., Gene Ther. 3(5):405-411 (1996); Tsurumi et al., Circulation 94(12):3281-3290 (1996) (incorporated herein by reference).

[1190] The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

[1191] The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, polynucleotides encoding albumin fusion proteins of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) Ann. NY Acad. Sci. 772:126-139 and Abdallah B. et al. (1995) Biol. Cell 85(1):1-7) which can be prepared by methods well known to those skilled in the art.

[1192] The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to

provide production of the desired polypeptide for periods of up to six months.

[1193] The polynucleotide construct can be delivered to the interstitial space of tissues within an animal, including muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

[1194] For the naked polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

[1195] The dose response effects of injected polynucleotide in muscle *in vivo* is determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the present invention is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

[1196] Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

[1197] After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 um cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A time course for fusion protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be used to extrapolate proper dosages and other treatment parameters in humans and other animals using naked DNA.

EXAMPLE 43: Biological Effects of Fusion Proteins of the Invention

Astrocyte and Neuronal Assays.

[1198] Albumin fusion proteins of the invention can be tested for activity in promoting the survival, neurite outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes. The selection of cortical cells for the bioassay is based on the prevalent expression of FGF-1 and FGF-2 in cortical structures and on the previously reported enhancement of cortical neuronal survival resulting from FGF-2 treatment. A thymidine incorporation assay, for example, can be used to elucidate an albumin fusion protein of the invention's activity on these cells.

[1199] Moreover, previous reports describing the biological effects of FGF-2 (basic FGF) on cortical or hippocampal neurons *in vitro* have demonstrated increases in both neuron survival and neurite outgrowth (Walicke et al., "Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension." *Proc. Natl. Acad. Sci. USA 83*:3012-3016. (1986), assay herein incorporated by reference in its entirety). However, reports from experiments done on PC-12 cells suggest that these two responses are not necessarily synonymous and may depend on not only which FGF is being tested but also on which receptor(s) are expressed on the target cells. Using the primary cortical neuronal culture paradigm, the ability of an albumin fusion protein of the invention to induce neurite outgrowth can be compared to the response achieved with FGF-2 using, for example, a thymidine incorporation assay.

Fibroblast and endothelial cell assays.

[1200] Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal

microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000 cells/well in a 96-well plate for one day in growth medium. The cells are then incubated for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA medium, the cells are incubated with the test fusion protein of the invention proteins for 3 days. Alamar Blue (Alamar Biosciences, Sacramento, CA) is added to each well to a final concentration of 10%. The cells are incubated for 4 hr. Cell viability is measured by reading in a CytoFluor fluorescence reader. For the PGE_2 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or fusion protein of the invention with or without IL-1α for 24 hours. The supernatants are collected and assayed for PGE_2 by EIA kit (Cayman, Ann Arbor, MI). For the IL-6 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or with or without an albumin fusion protein of the invention and/or IL-1α for 24 hours. The supernatants are collected and assayed for IL-6 by ELISA kit (Endogen, Cambridge, MA).

[1201] Human lung fibroblasts are cultured with FGF-2 or an albumin fusion protein of the invention for 3 days in basal medium before the addition of Alamar Blue to assess effects on growth of the fibroblasts. FGF-2 should show a stimulation at 10 - 2500 ng/ml which can be used to compare stimulation with the fusion protein of the invention.

Cell proliferation based on [3H]thymidine incorporation

[1202] The following [3H]Thymidine incorporation assay can be used to measure the effect of a Therapeutic proteins, e.g., growth factor proteins, on the proliferation of cells such as fibroblast cells, epithelial cells or immature muscle cells.

[1203] Sub-confluent cultures are arrested in G1 phase by an 18 h incubation in serum-free medium. Therapeutic proteins are then added for 24 h and during the last 4 h, the cultures are labeled with [3H]thymidine, at a final concentration of 0.33 μM (25 Ci/mmol, Amersham, Arlington Heights, IL). The incorporated [3H]thymidine is precipitated with ice-cold 10% trichloroacetic acid for 24 h. Subsequently, the cells are rinsed sequentially with ice-cold 10% trichloroacetic acid and then with ice-cold water. Following lysis in 0.5 M NaOH, the lysates and PBS rinses (500 ml) are pooled, and the amount of radioactivity is measured.

Parkinson Models.

[1204] The loss of motor function in Parkinson's disease is attributed to a deficiency of striatal dopamine resulting from the degeneration of the nigrostriatal dopaminergic projection neurons. An animal model for Parkinson's that has been extensively characterized involves the systemic administration of 1-methyl-4 phenyl 1,2,3,6-tetrahydropyridine (MPTP). In the CNS, MPTP is taken-up by astrocytes and catabolized by monoamine oxidase B to 1-methyl-4-phenyl pyridine (MPP^+) and released. Subsequently, MPP^+ is actively accumulated in dopaminergic neurons by the high-affinity reuptake transporter for dopamine. MPP^+ is then concentrated in mitochondria by the electrochemical gradient and selectively inhibits nicotidamide adenine diphosphate: ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and eventually generating oxygen radicals.

[1205] It has been demonstrated in tissue culture paradigms that FGF-2 (basic FGF) has trophic activity towards nigral dopaminergic neurons (Ferrari et al., Dev. Biol. 1989). Recently, Dr. Unsicker's group has demonstrated that administering FGF-2 in gel foam implants in the striatum results in the near complete protection of nigral dopaminergic neurons from the toxicity associated with MPTP exposure (Otto and Unsicker, J. Neuroscience, 1990).

[1206] Based on the data with FGF-2, an albumin fusion protein of the invention can be evaluated to determine whether it has an action similar to that of FGF-2 in enhancing dopaminergic neuronal survival *in vitro* and it can also be tested *in vivo* for protection of dopaminergic neurons in the striatum from the damage associated with MPTP treatment. The potential effect of an albumin fusion protein of the invention is first examined in vitro in a dopaminergic neuronal cell culture paradigm. The cultures are prepared by dissecting the midbrain floor plate from gestation day 14 Wistar rat embryos. The tissue is dissociated with trypsin and seeded at a density of 200,000 cells/cm^2 on polyornithine-laminin coated glass coverslips. The cells are maintained in Dulbecco's Modified Eagle's medium and F12 medium containing hormonal supplements (N1). The cultures are fixed with paraformaldehyde after 8 days in vitro and are processed for tyrosine hydroxylase, a specific marker for dopaminergic neurons, immunohistochemical staining. Dissociated cell cultures are prepared from embryonic rats. The culture medium is changed every third day and the factors are also added at that time.

[1207] Since the dopaminergic neurons are isolated from animals at gestation day 14, a developmental time which is past the stage when the dopaminergic precursor cells are proliferating, an increase in the number of tyrosine hydroxylase immunopositive neurons would represent an increase in the number of dopaminergic neurons surviving *in vitro*. Therefore, if a therapeutic protein of the invention acts to prolong the survival of dopaminergic neurons, it would suggest that the fusion protein may be involved in Parkinson's Disease.

EXAMPLE 44: Pancreatic Beta-Cell Transplantation Combination Therapy

[1208] Transplantation is a common form of treatment of autoimmune disease, especially when the target self tissue has been severely damaged.

For example, and not by way of limitation, pancreas transplantation and islet cell transplantation are common treatment options for IDDM (See, e.g., Stewart et al., Journal of Clinical Endocrinology & Metabolism 86 (3): 984-988 (2001); Brunicardi, Transplant. Proc. 28: 2138-40 (1996); Kendall & Robertson, Diabetes Metab. 22: 157-163 (1996); Hamano et al., Kobe J. Med. Sci. 42: 93-104 (1996); Larsen & Stratta, Diabetes Metab. 22: 139-146 (1996); and Kinkhabwala, et al., Am. J. Surg. 171: 516-520 (1996)). As with any transplantation method, transplantation therapies for autoimmune disease patients include treatments to minimize the risk of host rejection of the transplanted tissue. However, autoimmune disease involves the additional, independent risk that the pre-existing host autoimmune response which damaged the original self tissue will exert the same damaging effect on the transplanted tissue. Accordingly, the present invention encompasses methods and compositions for the treatment of autoimmune pancreatic disease using the albumin fusion proteins of the subject invetion in combination with immunomodulators/immunosuppressants in individuals undergoing transplantation therapy of the autoimmune disease.

[1209] In accordance with the invention, the albumin fusion-based compositions and formulations described above, are administered to prevent and treat damage to the transplanted organ, tissue, or cells resulting from the host individual's autoimmune response initially directed against the original self tissue. Administration may be carried out both prior and subsequent to transplantation in 2 to 4 doses each one week apart.

[1210] The following immunomodulators/immunosuppressants including, but not limited to, AI-401, CDP-571 (anti-TNF monoclonal antibody), CG-1088, Diamyd (diabetes vaccine), ICM3 (anti-ICAM-3 monoclonal antibody), linomide (Roquinimex), NBI-6024 (altered peptide ligand), TM-27, VX-740 (HMR-3480), caspase 8 protease inhibitors, thalidomide, hOKT3gamma1 (Ala-ala) (anti-CD3 monoclonal antibody), Oral Interferon-Alpha, oral lactobacillus, and LymphoStat-B™ can be used together with the albumin fusion therapeutics of the subject invention in islet cell or pancreas transplantation.

EXAMPLE 65: Identification and Cloning of VH and VL domains.

[1211] One method to identify and clone VH and VL domains from cell lines expressing a particular antibody is to perform PCR with VH and VL specific primers on cDNA made from the antibody expressing cell lines. Briefly, RNA is isolated from the cell lines and used as a template for RT-PCR designed to amplify the VH and VL domains of the antibodies expressed by the EBV cell lines. Cells may be lysed in the TRIzol® reagent (Life Technologies, Rockville, MD) and extracted with one fifth volume of chloroform. After addition of chloroform, the solution is allowed to incubate at room temperature for 10 minutes, and the centrifuged at 14,000 rpm for 15 minutes at 4°C in a tabletop centrifuge. The supernatant is collected and RNA is precipitated using an equal volume of isopropanol. Precipitated RNA is pelleted by centrifuging at 14,000 rpm for 15 minutes at 4°C in a tabletop centrifuge. Following centrifugation, the supernatant is discarded and washed with 75% ethanol. Follwing washing, the RNA is centrifuged again at 800 rpm for 5 minutes at 4°C. The supernatant is discarded and the pellet allowed to air dry. RNA is the dissolved in DEPC water and heated to 60°C for 10 minutes. Quantities of RNA can determined using optical density measurements.

[1212] cDNA may be synthesized, according to methods well-known in the art, from 1.5-2.5 micrograms of RNA using reverse transcriptase and random hexamer primers. cDNA is then used as a template for PCR amplification of VH and VL domains. Primers used to amplify VH and VL genes are shown in Table 7. Typically a PCR reaction makes use of a single 5' primer and a single 3' primer. Sometimes, when the amount of available RNA template is limiting, or for greater efficiency, groups of 5' and/or 3' primers may be used. For example, sometimes all five VH-5' primers and all JH3' primers are used in a single PCR reaction. The PCR reaction is carried out in a 50 microliter volume containing 1X PCR buffer, 2mM of each dNTP, 0.7 units of High Fidelity Taq polymerase, 5' primer mix, 3' primer mix and 7.5 microliters of cDNA. The 5' and 3' primer mix of both VH and VL can be made by pooling together 22 pmole and 28 pmole, respectively, of each of the individual primers. PCR conditions are: 96°C for 5 minutes; followed by 25 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 1 minute; followed by an extension cycle of 72°C for 10 minutes. After the reaction is completed, sample tubes are stored 4°C.

Table 7: Primer Sequences Used to Amplify VH and VL domains.

Primer name	SEQ ID NO	Primer Sequence (5'-3')
VH Primers		
Hu VH1-5'	62	CAGGTGCAGCTGGTGCAGTCTGG
Hu VH2-5'	63	CAGGTCAACTTAAGGGAGTCTGG
Hu VH3-5'	64	GAGGTGCAGCTGGTGGAGTCTGG
Hu VH4-5'	65	CAGGTGCAGCTGCAGGAGTCGGG
Hu VH5-5'	66	GAGGTGCAGCTGTTGCAGTCTGC
Hu VH6-5'	67	CAGGTACAGCTGCAGCAGTCAGG
Hu JH1,2-5'	68	TGAGGAGACGGTGACCAGGGTGCC
Hu JH3-5'	69	TGAAGAGACGGTGACCATTGTCCC
Hu JH4,5-5'	70	TGAGGAGACGGTGACCAGGGTTCC
Hu JH6-5'	71	TGAGGAGACGGTGACCGTGGTCCC

VL Primers

Hu Vkappa1-5'	72	GACATCCAGATGACCCAGTCTCC
Hu Vkappa2a-5'	73	GATGTTGTGATGACTCAGTCTCC
Hu Vkappa2b-5'	74	GATATTGTGATGACTCAGTCTCC
Hu Vkappa3-5'	75	GAAATTGTGTTGACGCAGTCTCC
Hu Vkappa4-5'	76	GACATCGTGATGACCCAGTCTCC
Hu Vkappa5-5'	77	GAAACGACACTCACGCAGTCTCC
Hu Vkappa6-5'	78	GAAATTGTGCTGACTCAGTCTCC
Hu Vlambda1-5'	79	CAGTCTGTGTTGACGCAGCCGCC
Hu Vlambda2-5'	80	CAGTCTGCCCTGACTCAGCCTGC
Hu Vlambda3-5'	81	TCCTATGTGCTGACTCAGCCACC
Hu Vlambda3b-5'	82	TCTTCTGAGCTGACTCAGGACCC
Hu Vlambda4-5'	83	CACGTTATACTGACTCAACCGCC
Hu Vlambda5-5'	84	CAGGCTGTGCTCACTCAGCCGTC
Hu Vlambda6-5'	85	AATTTTATGCTGACTCAGCCCCA
Hu Jkappa1-3'	86	ACGTTTGATTTCCACCTTGGTCCC
Hu Jkappa2-3'	87	ACGTTTGATCTCCAGCTTGGTCCC
Hu Jkappa3-3'	88	ACGTTTGATATCCACTTTGGTCCC
Hu Jkappa4-3'	89	ACGTTTGATCTCCACCTTGGTCCC
Hu Jkappa5-3'	90	ACGTTTAATCTCCAGTCGTGTCCC
Hu Jlambda1-3'	91	CAGTCTGTGTTGACGCAGCCGCC
Hu Jlambda2-3'	92	CAGTCTGCCCTGACTCAGCCTGC
Hu Jlambda3--3'	93	TCCTATGTGCTGACTCAGCCACC
Hu Jlambda3b-3'	94	TCTTCTGAGCTGACTCAGGACCC
Hu Jlambda4-3'	95	CACGTTATACTGACTCAACCGCC
Hu Jlambda5-3'	96	CAGGCTGTGCTCACTCAGCCGTC
Hu Jlambda6-3'	97	AATTTTATGCTGACTCAGCCCCA

PCR samples are then electrophoresed on a 1.3% agarose gel. DNA bands of the expected sizes (~506 base pairs for VH domains, and 344 base pairs for VL domains) can be cut out of the gel and purified using methods well known in the art. Purified PCR products can be ligated into a PCR cloning vector (TA vector from Invitrogen Inc., Carlsbad, CA). Individual cloned PCR products can be isolated after transfection of E. coli and blue/white color selection. Cloned PCR products may then be sequenced using methods commonly known in the art.

[1213] The PCR bands containing the VH domain and the VL domains can also be used to create full-length Ig expression vectors. VH and VL domains can be cloned into vectors containing the nucleotide sequences of a heavy (e.g., human IgG1 or human IgG4) or light chain (human kappa or human lambda) constant regions such that a complete heavy or light chain molecule could be expressed from these vectors when transfected into an appropriate host cell. Further, when cloned heavy and light chains are both expressed in one cell line (from either one or two vectors), they can assemble into a complete functional antibody molecule that is secreted into the cell culture medium. Methods using polynucleotides encoding VH and VL antibody domain to generate expression vectors that encode complete antibody molecules are well known within the art.

EXAMPLE 66: Preparation of HA-cytokine or HA-growth factor fusion proteins (such as NGF, BDNFa, BDNFb and BDNFc).

[1214] The cDNA for the cytokine or growth factor of interest, such as NGF, can be isolated by a variety of means including from cDNA libraries, by RT-PCR and by PCR using a series of overlapping synthetic oligonucleotide primers, all using standard methods. The nucleotide sequences for all of these proteins are known and available. The cDNA can be tailored at the 5' and 3' ends to generate restriction sites, such that oligonucleotide linkers can be used, for cloning of the cDNA into a vector containing the cDNA for HA. This can be at the N or C-terminus with or without the use of a spacer sequence. NGF (or other cytokine) cDNA is cloned into a vector such as pPPC0005 (Figure 2), pScCHSA, pScNHSA , or pC4:HSA from which the complete expression cassette is then excised and inserted into the plasmid pSAC35 to allow the expression of the albumin fusion protein in yeast. The albumin fusion protein secreted from the yeast can then be collected and purified from the media and tested for its biological activity. For expression in mammalian cell lines, a similar procedure is adopted except that the expression cassette used employs a mammalian promoter, leader sequence and terminator (See Example 1). This expression cassette is then excised and inserted into a plasmid suitable for the transfection of mammalian cell lines.

EXAMPLE 67: Preparation of HA-IFN fusion proteins (such as IFNα).

[1215] The cDNA for the interferon of interest such as IFNα can be isolated by a variety of means including but not exclusively, from cDNA

libraries, by RT-PCR and by PCR using a series of overlapping synthetic oligonucleotide primers, all using standard methods. The nucleotide sequences for interferons, such as IFNα are known and available, for instance, in U.S. Patents 5,326,859 and 4,588,585, in EP 32 134, as well as in public databases such as GenBank. The cDNA can be tailored at the 5' and 3' ends to generate restriction sites, such that oligonucleotide linkers can be used to clone the cDNA into a vector containing the cDNA for HA. This can be at the N or C-terminus of the HA sequence, with or without the use of a spacer sequence. The IFNα (or other interferon) cDNA is cloned into a vector such as pPPC0005 (Figure 2), pScCHSA, pScNHSA , or pC4:HSA from which the complete expression cassette is then excised and inserted into the plasmid pSAC35 to allow the expression of the albumin fusion protein in yeast. The albumin fusion protein secreted from the yeast can then be collected and purified from the media and tested for its biological activity. For expression in mammalian cell lines a similar procedure is adopted except that the expression cassette used employs a mammalian promoter, leader sequence and terminator (See Example 1). This expression cassette is then excised and inserted into a plasmid suitable for the transfection of mammalian cell lines.

Maximum protein recovery from vials

[1216] The albumin fusion proteins of the invention have a high degree of stability even when they are packaged at low concentrations. In addition, in spite of the low protein concentration, good fusion-protein recovery is observed even when the aqueous solution includes no other protein added to minimize binding to the vial walls. The recovery of vial-stored HA-IFN solutions was compared with a stock solution. 6 or 30 μg/ml HA-IFN solutions were placed in vials and stored at 4°C. After 48 or 72 hrs a volume originally equivalent to 10 ng of sample was removed and measured in an IFN sandwich ELISA. The estimated values were compared to that of a high concentration stock solution. As shown, there is essentially no loss of the sample in these vials, indicating that addition of exogenous material such as albumin is not necessary to prevent sample loss to the wall of the vials

In vivo stability and bioavailability of HA-α-IFN fusions

[1217] To determine the in vivo stability and bioavailability of a HA-α-IFN fusion molecule, the purified fusion molecule (from yeast) was administered to monkeys. Pharmaceutical compositions formulated from HA-α-IFN fusions may account for the extended serum half-life and bioavailability. Accordingly, pharmaceutical compositions may be formulated to contain lower dosages of alpha-interferon activity compared to the native alpha-interferon molecule.

[1218] Pharmaceutical compositions containing HA-α-IFN fusions may be used to treat or prevent disease in patients with any disease or disease state that can be modulated by the administration of α-IFN. Such diseases include, but are not limited to, hairy cell leukemia, Kaposi's sarcoma, genital and anal warts, chronic hepatitis B, chronic non-A, non-B hepatitis, in particular hepatitis C, hepatitis D, chronic myelogenous leukemia, renal cell carcinoma, bladder carcinoma, ovarian and cervical carcinoma, skin cancers, recurrent respirator papillomatosis, non-Hodgkin's and cutaneous T-cell lymphomas, melanoma, multiple myeloma , AIDS, multiple sclerosis, glioblastoma, etc. (see Interferon Alpha, In: AHFS Drug Information, 1997.

[1219] Accordingly, the invention includes pharmaceutical compositions containing a HA-α-IFN fusion protein, polypeptide or peptide formulated with the proper dosage for human administration. The invention also includes methods of treating patients in need of such treatment comprising at least the step of administering a pharmaceutical composition containing at least one HA-α-IFN fusion protein, polypeptide or peptide.

Bifunctional HA-α-IFN fusions

[1220] A HA-α-IFN expression vector may be modified to include an insertion for the expression of bifunctional HA-α-IFN fusion proteins. For instance, the cDNA for a second protein of interest may be inserted in frame downstream of the "rHA-IFN" sequence after the double stop codon has been removed or shifted downstream of the coding sequence.

[1221] In one version of a bifunctional HA-α-IFN fusion protein, an antibody or fragment against B-lymphocyte stimulator protein (GenBank Acc 4455139) or polypeptide may be fused to one end of the HA component of the fusion molecule. This bifunctional protein is useful for modulating any immune response generated by the α-IFN component of the fusion.

EXAMPLE 68: Preparation of HA-hormone fusion protein

[1222] The cDNA for the hormone of interest can be isolated by a variety of means including but not exclusively, from cDNA libraries, by RT-PCR and by PCR using a series of overlapping synthetic oligonucleotide primers, all using standard methods. The nucleotide sequences for all of these proteins are known and available, for instance, in public databases such as GenBank. The cDNA can be tailored at the 5' and 3' ends to generate restriction sites, such that oligonucleotide linkers can be used, for cloning of the cDNA into a vector containing the cDNA for HA. This can be at the N or C-terminus with or without the use of a spacer sequence. The hormone cDNA is cloned into a vector such as pPPC0005 (Figure 2), pScCHSA, pScNHSA , or pC4:HSA from which the complete expression cassette is then excised and inserted into the plasmid pSAC35 to allow the expression of the albumin fusion protein in yeast. The albumin fusion protein secreted from the yeast can then be collected and purified from the media and tested for its biological activity. For expression in mammalian cell lines a similar procedure is adopted except that the expression cassette used employs a mammalian promoter, leader sequence and terminator (See Example 1). This expression cassette is then excised and inserted into a plasmid suitable for the transfection of mammalian cell lines.

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EXAMPLE 69: Preparation of HA-soluble receptor or HA-binding protein fusion protein.

[1223] The cDNA for the soluble receptor or binding protein of interest can be isolated by a variety of means including but not exclusively, from cDNA libraries, by RT-PCR and by PCR using a series of overlapping synthetic oligonucleotide primers, all using standard methods. The nucleotide sequences for all of these proteins are known and available, for instance, in GenBank. The cDNA can be tailored at the 5' and 3' ends to generate restriction sites, such that oligonucleotide linkers can be used, for cloning of the cDNA into a vector containing the cDNA for HA. This can be at the N or C-terminus with or without the use of a spacer sequence. The receptor cDNA is cloned into a vector such as pPPC0005 (Figure 2), pScCHSA, pScNHSA , or pC4:HSA from which the complete expression cassette is then excised and inserted into the plasmid pSAC35 to allow the expression of the albumin fusion protein in yeast. The albumin fusion protein secreted from the yeast can then be collected and purified from the media and tested for its biological activity. For expression in mammalian cell lines a similar procedure is adopted except that the expression cassette used employs a mammalian promoter, leader sequence and terminator (See Example 1). This expression cassette is then excised and inserted into a plasmid suitable for the transfection of mammalian cell lines.

EXAMPLE 70: Preparation of HA-growth factors.

[1224] The cDNA for the growth factor of interest can be isolated by a variety of means including but not exclusively, from cDNA libraries, by RT-PCR and by PCR using a series of overlapping synthetic oligonucleotide primers, all using standard methods (see GenBank Acc. No.NP_000609). The cDNA can be tailored at the 5' and 3' ends to generate restriction sites, such that oligonucleotide linkers can be used, for cloning of the cDNA into a vector containing the cDNA for HA. This can be at the N or C-terminus with or without the use of a spacer sequence. The growth factor cDNA is cloned into a vector such as pPPC0005 (Figure 2), pScCHSA, pScNHSA , or pC4:HSA from which the complete expression cassette is then excised and inserted into the plasmid pSAC35 to allow the expression of the albumin fusion protein in yeast. The albumin fusion protein secreted from the yeast can then be collected and purified from the media and tested for its biological activity. For expression in mammalian cell lines a similar procedure is adopted except that the expression cassette used employs a mammalian promoter, leader sequence and terminator (See Example 1). This expression cassette is then excised and inserted into a plasmid suitable for the transfection of mammalian cell lines.

EXAMPLE 71: Preparation of HA-single chain antibody fusion proteins.

[1225] Single chain antibodies are produced by several methods including but not limited to: selection from phage libraries, cloning of the variable region of a specific antibody by cloning the cDNA of the antibody and using the flanking constant regions as the primer to clone the variable region, or by synthesizing an oligonucleotide corresponding to the variable region of any specific antibody. The cDNA can be tailored at the 5' and 3' ends to generate restriction sites, such that oligonucleotide linkers can be used, for cloning of the cDNA into a vector containing the cDNA for HA. This can be at the N or C-terminus with or without the use of a spacer sequence. The cell cDNA is cloned into a vector such as pPPC0005 (Figure 2), pScCHSA, pScNHSA , or pC4:HSA from which the complete expression cassette is then excised and inserted into the plasmid pSAC35 to allow the expression of the albumin fusion protein in yeast.

[1226] In fusion molecules of the invention, the V_H and V_L can be linked by one of the following means or a combination thereof: a peptide linker between the C-terminus of the V_H and the N-terminus of the V_L; a Kex2p protease cleavage site between the V_H and V_L such that the two are cleaved apart upon secretion and then self associate; and cystine residues positioned such that the V_H and V_L can form a disulphide bond between them to link them together. An alternative option would be to place the V_H at the N-terminus of HA or an HA domain fragment and the V_L at the C-terminus of the HA or HA domain fragment.

[1227] The albumin fusion protein secreted from the yeast can then be collected and purified from the media and tested for its activity. For expression in mammalian cell lines a similar procedure is adopted except that the expression cassette used employs a mammalian promoter, leader sequence and terminator (See Example 1). This expression cassette is then excised and inserted into a plasmid suitable for the transfection of mammalian cell lines. The antibody produced in this manner can be purified from media and tested for its binding to its antigen using standard immunochemical methods.

EXAMPLE 72: Preparation of HA-cell adhesion molecule fusion proteins.

[1228] The cDNA for the cell adhesion molecule of interest can be isolated by a variety of means including but not exclusively, from cDNA libraries, by RT-PCR and by PCR using a series of overlapping synthetic oligonucleotide primers, all using standard methods. . The nucleotide sequences for the known cell adhesion molecules are known and available, for instance, in GenBank. The cDNA can be tailored at the 5' and 3' ends to generate restriction sites, such that oligonucleotide linkers can be used, for cloning of the cDNA into a vector containing the cDNA for HA. This can be at the N or C-terminus with or without the use of a spacer sequence. The cell adhesion molecule cDNA is cloned into a vector such as pPPC0005 (Figure 2), pScCHSA, pScNHSA , or pC4:HSA from which the complete expression cassette is then excised and inserted into the plasmid pSAC35 to allow the expression of the albumin fusion protein in yeast. The albumin fusion protein secreted from the yeast can then be collected and purified from the media and tested for its biological activity. For expression in mammalian cell lines a similar procedure is adopted except that the expression cassette used employs a mammalian promoter, leader sequence and terminator (See Example 1). This expression cassette is then excised and inserted into a plasmid suitable for the transfection of mammalian cell lines.

EXAMPLE 73: Preparation of inhibitory factors and peptides as HA fusion proteins (such as HA-antiviral, HA-antibiotic, HA-enzyme inhibitor and HA-anti-allergic proteins).

[1229] The cDNA for the peptide of interest such as an antibiotic peptide can be isolated by a variety of means including but not exclusively, from cDNA libraries, by RT-PCR and by PCR using a series of overlapping synthetic oligonucleotide primers, all using standard methods. The cDNA can be tailored at the 5' and 3' ends to generate restriction sites, such that oligonucleotide linkers can be used, for cloning of the cDNA into a vector containing the cDNA for HA. This can be at the N or C-terminus with or without the use of a spacer sequence. The peptide cDNA is cloned into a vector such as pPPC0005 (Figure 2), pScCHSA, pScNHSA , or pC4:HSA from which the complete expression cassette is then excised and inserted into the plasmid pSAC35 to allow the expression of the albumin fusion protein in yeast. The albumin fusion protein secreted from the yeast can then be collected and purified from the media and tested for its biological activity. For expression in mammalian cell lines a similar procedure is adopted except that the expression cassette used employs a mammalian promoter, leader sequence and terminator (See Example 1). This expression cassette is then excised and inserted into a plasmid suitable for the transfection of mammalian cell lines.

EXAMPLE 74: Preparation of targeted HA fusion proteins.

[1230] The cDNA for the protein of interest can be isolated from cDNA library or can be made synthetically using several overlapping oligonucleotides using standard molecular biology methods. The appropriate nucleotides can be engineered in the cDNA to form convenient restriction sites and also allow the attachment of the protein cDNA to albumin cDNA. Also a targeting protein or peptide cDNA such as single chain antibody or peptides, such as nuclear localization signals, that can direct proteins inside the cells can be fused to the other end of albumin. The protein of interest and the targeting peptide is cloned into a vector such as pPPC0005 (Figure 2), pScCHSA, pScNHSA , or pC4:HSA which allows the fusion with albumin cDNA. In this manner both N- and C-terminal end of albumin are fused to other proteins. The fused cDNA is then excised from pPPC0005 and is inserted into a plasmid such as pSAC35 to allow the expression of the albumin fusion protein in yeast. All the above procedures can be performed using standard methods in molecular biology. The albumin fusion protein secreted from yeast can be collected and purified from the media and tested for its biological activity and its targeting activity using appropriate biochemical and biological tests.

EXAMPLE 75: Preparation of HA-enzyme fusions.

[1231] The cDNA for the enzyme of interest can be isolated by a variety of means including but not exclusively, from cDNA libraries, by RT-PCR and by PCR using a series of overlapping synthetic oligonucleotide primers, all using standard methods. The cDNA can be tailored at the 5' and 3' ends to generate restriction sites, such that oligonucleotide linkers can be used, for cloning of the cDNA into a vector containing the cDNA for HA. This can be at the N or C-terminus with or without the use of a spacer sequence. The enzyme cDNA is cloned into a vector such as pPPC0005 (Figure 2), pScCHSA, pScNHSA, or pC4:HSA from which the complete expression cassette is then excised and inserted into the plasmid pSAC35 to allow the expression of the albumin fusion protein in yeast. The albumin fusion protein secreted from the yeast can then be collected and purified from the media and tested for its biological activity. For expression in mammalian cell lines a similar procedure is adopted except that the expression cassette used employs a mammalian promoter, leader sequence and terminator (See Example 1). This expression cassette is then excised and inserted into a plasmid suitable for the transfection of mammalian cell lines.

EXAMPLE 76: Construct ID 2053, IFNb-HSA, Generation.

[1232] Construct ID 2053, pEE12.1:IFNb.HSA, comprises DNA encoding an IFNb albumin fusion protein which has the full-length IFNb protein including the native IFNb leader sequence fused to the amino-terminus of the mature form of HSA in the NS0 expression vector pEE12.1.

Cloning of IFNb cDNA

[1233] The polynucleotide encoding IFNb was PCR amplified using primers IFNb-1 and IFNb-2, described below, cut *with Bam HI/Cla I*, and ligated into *Bam HI/Cla I* cut pC4:HSA, resulting in construct 2011. The *Eco* RI/*Eco* RI fragment from Construct ID # 2011 was subcloned into the *Eco RI site of pEE12.1 generating construct ID #2053 which which comprises DNA encoding an albumin fusion protein containing the leader* sequence and the mature form of IFNb, followed by the mature HSA protein.

[1234] Two oligonucleotides suitable for PCR amplification of the polynucleotide encoding the full-length of IFNb, IFNb-1 and IFNb-2, were synthesized:

IFNb-1: 5'-GCGCGGATCC*G*AATTC*CGCCGCCATG*ACCAACAAGTGTCTCCTCCAAATTGCTCTCCTGTTGTGCTT CTCCACTACAGCTCTTTCCATGAGCTACAACTTGCTTGG-3' (SEQ ID NO:107)

IFNb-2: 5'-GCGCGCATCGATGAGCAACCTCACTCTTGTGTGCATCGTTTCGGA GGTAACCTGT-3' (SEQ ID NO:108)

[1235] The IFNb-1 primer incorporates a *Bam* HI cloning site (shown underlined), an *Eco* RI cloning site, and a Kozak sequence (shown in italics), followed by 80 nucleotides encoding the first 27 amino acids of the full-length form of IFNb. In IFNb-2, the *Cla* I site (shown underlined) and the DNA following it are the reverse complement of DNA encoding the first 10 amino acids of the mature HSA protein (SEQ ID NO:1) and the last 18 nucleotides are the reverse complement of DNA encoding the last 6 amino acid residues of IFNb (see Example 2). A PCR amplimer was generated using these primers, purified, digested with *Bam* HI and *Cla* I restriction enzymes, and cloned into the *Bam* HI and *Cla* I sites of the pC4:HSA vector. After the sequence was confirmed, an *Eco* RI fragment containing the IFNb albumin fusion protein expression cassette was subcloned into *Eco* RI digested pEE12.1.

[1236] Further, analysis of the N-terminus of the expressed albumin fusion protein by amino acid sequencing can confirm the presence of the expected IFNb sequence (see below).

[1237] IFNb albumin fusion proteins of the invention preferably comprise the mature form of HSA , i.e., Asp-25 to Leu-609, fused to either the N- or C- terminus of the mature form of IFNb, i.e., Met-22 to Asn-187. In one embodiment of the invention, IFNb albumin fusion proteins of the invention further comprise a signal sequence which directs the nascent fusion polypeptide in the secretory pathways of the host used for expression. In a further preferred embodiment, the signal peptide encoded by the signal sequence is removed, and the mature IFNb albumin fusion protein is secreted directly into the culture medium. IFNb albumin fusion proteins of the invention may comprise heterologous signal sequences including, but not limited to, MAF, INV, Ig, Fibulin B, Clusterin, Insulin-Like Growth Factor Binding Protein 4, variant HSA leader sequences including, but not limited to, a chimeric HSA/MAF leader sequence, or other heterologous signal sequences known in the art. In a preferred embodiment, IFNb albumin fusion proteins of the invention comprise the native IFNb. In further preferred embodiments, the IFNb albumin fusion proteins of the invention further comprise an N-terminal methionine residue. Polynucleotides encoding these polypeptides, including fragments and/or variants, are also encompassed by the invention.

Expression and Purification of Construct ID 2053.

Expression in murine myeloma NS0 cell-lines.

[1238] Construct ID # 2053, pBR12.1:IFNb-HSA , was electroporated into NS0 cells by methods known in the art (see Example 6).

Purification from NS0 cell supernatant.

[1239] Purification of IFNb-HSA from NS0 cell supernatant may involve Q-Sepharose anion exchange chromatography at pH 7.4 using a NaCl gradient from 0 to 1 M in 20 mM Tris-HCl, followed by Poros PI 50 anion exchange chromatography at pH 6.5 with a sodium citrate gradient from 5 to 40 mM, and diafiltrating for 6 DV into 10 mM citrate, pH 6.5 and 140 mM NaCl, the final buffer composition. N-terminal sequencing should yield the sequence MSYNLL which is the amino terminus of the mature form of IFNb. The protein has an approximate MW of 88.5 kDa.

[1240] For larger scale purification, e.g., 50 L of NS0 cell supernatant can be concentrated into ~8 to 10 L. The concentrated sample can then be passed over the Q-Sepharose anion exchange column (10 x 19 cm, 1.5 L) at pH 7.5 using a step elution consisting of 50 mM NaOAc, pH 6.0 and 150 mM NaCl. The eluted sample can then be virally inactivated with 0.75% Triton-X 100 for 60 min at room temperature. SDR-Reverse Phase chromatography (10 cm x 10 cm, 0.8 L) can then be employed at pH 6.0 with 50 mM NaOAc and 150 mM NaCl, or alternatively, the sample can be passed over an SP-sepharose column at pH 4.8 using a step elution of 50 mM NaOAc, pH 6.0, and 150 mM NaCl. DV 50 filtration would follow to remove any viral content. Phenyl-650M chromatography (20 cm x 12 cm, 3.8 L) at pH 6.0 using a step elution consisting of 350 mM $(NH_4)_2SO_4$ and 50 mM NaOAc, or alternatively consisting of 50 mM NaOAc pH 6.0, can follow. Diafiltration for 6-8 DV will allow for buffer exchange into the desired final formulation buffer of either 10 mM Na_2HPO_4 + 58 mM sucrose + 120 mM NaCl, pH 7.2 or 10 mM citrate, pH 6.5, and 140 mM NaCl or 25 mM Na_2HPO_4, 100 mM NaCl, pH 7.2.

The activity of IFNb can be assayed using an in vitro ISRE-SEAP assay.

[1241] All type I Interferon proteins signal through a common receptor complex and a similar Jak/STAT signaling pathway that culminates in the activation of Interferon, "IFN", responsive genes through the Interferon Sequence Responsive Element, "ISRE". A convenient assay for type I IFN activity is a promoter-reporter based assay system that contains multiple copies of the ISRE element fused to a downstream reporter gene. A stable HEK293 cell-line can be generated and contains a stably integrated copy of an ISRE-SEAP reporter gene that is extremely sensitive to type I IFNs and displays linearity over 5 logs of concentration.

Method of Screening of IFNb-HSA NS0 stable clones.

[1242] Construct 2053 was electroporated into NS0 cells as described in Example 6. The NS0 cells transfected with construct ID # 2053 were screened for activity by testing conditioned growth media in the ISRE-SEAP assay. The ISRE-SEAP/293F reporter cells were plated at 3 x 10^4 cell/well in 96-well, poly-D-lysine coated, plates, one day prior to treatment. Reporter cells were treated with various dilutions (including but not limited to 1:500 and 1:5000) of conditioned supernatant or purified preparations of IFNb albumin fusion protein encoded by construct ID 2053 or rhIFNb as a control. The reporter cells were then incubated for 24 hours prior to removing 40 µL for use in the SEAP Reporter Gene Chemiluminescent Assay (Roche catalog # 1779842). Recombinant human Interferon beta, "rhIFNb" (Biogen), was used as a positive control.

Result

[1243] The purified preparation of NS0 expressed IFNb-HSA had a greater EC50 of 9.3 x 10^{-9} g/mL than rhIFNb (Biogen) which had an EC50 of 1.8 x 10^{-10} g/mL (see Figure 4).

In vivo Induction of OAS by an Interferon.

Method

[1244] The OAS enzyme, 2'-5'- OligoAdenylate Synthetase, is activated at the transcriptional level by interferon in response to antiviral infection. The effect of interferon constructs can be measured by obtaining blood samples from treated monkeys and analyzing these samples for transcriptional activation of two OAS mRNA, p41 and p69. A volume of 0.5 mL of whole blood can be obtained from 4 animals per group at 7 different time points, day 0, day 1, day 2, day 4, day 8, day 10, and day 14 per animal. The various groups may include injection of vehicle control,

intravenous and/or subcutaneous injection of either 30 μg/kg and/or 300 μg/kg IFN albumin fusion protein on day 1, and subcutaneous injection of 40 μg/kg of Interferon alpha (Schering-Plough) as a positive control on days 1, 3, and 5. The levels of the p41 and the p69 mRNA transcripts can be determined by real-time quantitative PCR (Taqman) using probes specific for p41-OAS and p69-OAS. OAS mRNA levels can be quantitated relative to 18S ribosomal RNA endogenous control.

In vivo induction of OAS by Interferon beta albumin fusion encoded by construct ID 2053.

Method

[1245] The activity of the HSA-IFNb fusion protein encoded by construct 2053 can be assayed in the *in vivo* OAS assay as previously described above under subsection heading, "*In vivo* induction of OAS by an Interferon".

EXAMPLE 77: Indications for IFNb albumin fusion proteins.

[1246] IFN beta albumin fusion proteins (including, but not limited to, those encoded by construct 2053) can be used to treat, prevent, ameliorate and/or detect multiple sclerosis. Other indications include, but are not limited to Viral infections including Severe Acute Respiratory Syndrome (SARS) and other coronavirus infections; filoviruses, including but not limited to Ebola viruses and Marburg virus; Arenaviruses, including but not limited to Pichende virus, Lassa virus, Junin virus, Machupo virus, Guanarito virus; and lymphocytic choriomeningitis virus (LCMV); Bunyaviruses, including but not limited to Punta toro virus, Crimean-Congo hemorrhagic fever virus, sandfly fever viruses, Rift Valley fever virus, La Crosse virus, and hantaviruses; Flavivirues, including but not limited to Yellow Fever, Banzi virus, West Nile virus, Dengue viruses, Japanese Encephalitis virus, Tick-borne encephalitis, Omsk Hemorrhagic Fever, and Kyasanur Forest Disease virus; Togaviruses, including but not limited to Venezuelan, eastern, and western equine encephalitis viruses, Ross River virus, and Rubella virus; Orthopox viruses, including but not limited to Vaccinia, Cowpox, Smallpox, and Monkeypox; Herpesviruses; FluA/B; Respiratory Sincytial virus (RSV); parafiu; measles; rhinoviruses; adenoviruses; Semliki Forest virus; Viral Hemorrhagic fevers; Rhabdoviruses; Paramyxoviruses, including but not limited to Nipah virus and Hendra virus; and other viral agents identified by the U.S. Centers for Disease Control and Prevention as high-priority disease agents (*i.e.*, Category A, B, and C agents; see, e.g., Moran, Emerg. Med. Clin. North. Am. 2002; 20(2):311-30 and Darling et al., Emerg. Med. Clin. North Am. 2002;20(2)-273-309).

EXAMPLE 78: Construct ID 2249, IFNa2-HSA, Generation.

[1247] Construct ID 2249, pSAC35:IFNa2.HSA, comprises DNA encoding an IFNa2 albumin fusion protein which has the HSA chimeric leader sequence, followed by the mature form of IFNa2 protein, i.e., C1-E165, fused to the amino-terminus of the mature form of HSA in the yeast *S. cerevisiae* expression vector pSAC35.

Cloning of IFNa2 cDNA

[1248] The polynucleotide encoding IFNa2 was PCR amplified using primers IFNa2-1 and IFNa2-2, described below. The PCR amplimer was cut with *Sal I/Cla* I, and ligated into *Xho I/Cla* I cut pScCHSA. Construct ID #2249 encodes an albumin fusion protein containing the chimeric leader sequence of HSA, the mature form of IFNa2, followed by the mature HSA protein.

[1249] Two oligonucleotides suitable for PCR amplification of the polynucleotide encoding the mature form of IFNa2, IFNa2-1 and IFNa2-2, were synthesized:

IFNa2-1: 5'-CGCGCGCGTTCGACAAAAGATGTGATCTGCCTCAAACCCACA-3' (SEQ ID NO:109)

IFNa2-2: 5'-GCGCGCATCGATGAGCAACCTCACTCTTGTGTGCATCTTCCTTACTTCTTAAACTTTCT-3' (SEQ ID NO:110)

[1250] The IFNa2-1 primer incorporates a *Sal* I cloning site (shown underlined), nucleotides encoding the last three amino acid residues of the chimeric HSA leader sequence, as well as 22 nucleotides (shown in bold) encoding the first 7 amino acid residues of the mature form of IFNa2. In IFNa2-2, the *Cla* I site (shown underlined) and the DNA following it are the reverse complement of DNA encoding the first 10 amino acids of the mature HSA protein and the last 22 nucleotides (shown in bold) are the reverse complement of DNA encoding the last 7 amino acid residues of IFNa2 (see Example 2). A PCR amplimer of IFNa2-HSA was generated using these primers, purified, digested with *Sal* I and *Cla* I restriction enzymes, and cloned into the *Xho* I and *Cla* I sites of the pScCHSA vector. After the sequence was confirmed, the expression cassette encoding this IFNa2 albumin fusion protein was subcloned into *Not* I digested pSAC35.

[1251] Further, analysis of the N-terminus of the expressed albumin fusion protein by amino acid sequencing can confirm the presence of the expected IFNa2 sequence (see below).

[1252] Other IFNa2 albumin fusion proteins using different leader sequences have been constructed by methods known in the art (see Example 2). Examples of the various leader sequences include, but are not limited to, invertase "INV" (constructs 2343 and 2410) and mating alpha factor "MAF" (construct 2366). These IFNa2 albumin fusion proteins can be subcloned into mammalian expression vectors such as pC4 (constructs 2382) and pEE12.1 as described previously (see Example 5). IFNa2 albumin fusion proteins with the therapeutic portion fused C-terminus to HSA can also be constructed (construct 2381).

[1253] IFNa2 albumin fusion proteins of the invention preferably comprise the mature form of HSA , i.e., Asp-25 to Leu-609, fused to either the N- or C- terminus of the mature form of IFNa2, i.e., Cys-1 to Glu-165. In one embodiment of the invention, IFNa2 albumin fusion proteins of the invention further comprise a signal sequence which directs the nascent fusion polypeptide in the secretory pathways of the host used for expression.

In a further preferred embodiment, the signal peptide encoded by the signal sequence is removed, and the mature IFNα2 albumin fusion protein is secreted directly into the culture medium. IFNα2 albumin fusion proteins of the invention may comprise heterologous signal sequences including, but not limited to, MAF, INV, Ig, Fibulin B, Clusterin, Insulin-Like Growth Factor Binding Protein 4, variant HSA leader sequences including, but not limited to, a chimeric HSA/MAF leader sequence, or other heterologous signal sequences known in the art. In a preferred embodiment, IFNα2 albumin fusion proteins of the invention comprise the native IFNα2. In further preferred embodiments, the IFNα2 albumin fusion proteins of the invention further comprise an N-terminal methionine residue. Polynucleotides encoding these polypeptides, including fragments and/or variants, are also encompassed by the invention.

Expression and Purification of Construct ID 2249.

Expression in yeast S. cerevisiae.

[1254] Transformation of construct 2249 into yeast *S. cerevisiae* strain BXP10 was carried out by methods known in the art (see Example 3). Cells can be collected at stationary phase after 72 hours of growth. Supernatants are collected by clarifying cells at 3000g for 10 min. Expression levels are examined by immunoblot detection with anti-HSA serum (Kent Laboratories) or as the primary antibody. The IFNα2 albumin fusion protein of approximate molecular weight of 88.5 kDa can be obtained.

Purification from yeast S. cerevisiae cell supernatant.

[1255] The cell supernatant containing IFNα2 albumin fusion protein expressed from construct ID #2249 in yeast *S. cerevisiae* cells can be purified either small scale over a Dyax peptide affinity column (see Example 4) or large scale by following 5 steps: diafiltration, anion exchange chromatography using DEAE-Sepharose Fast Flow column, hydrophobic interaction chromatography (HIC) using Butyl 650S column, cation exchange chromatography using an SP-Sepharose Fast Flow column or a Blue-Sepharose chromatography, and high performance chromatography using Q-sepharose high performance column chromatography (see Example 4). The IFNα2 albumin fusion protein may elute from the DEAE-Sepharose Fast Flow column with 100 – 250 mM NaCl, from the SP-Sepharose Fast Flow column with 150 – 250 mM NaCl, and from the Q-Sepharose High Performance column at 5 – 7.5 mS/cm. N-terminal sequencing should yield the sequence CDLPQ (SEQ ID NO:98) which corresponds to the mature form of IFNα2.

The activity of IFNα2 can be assayed using an in vitro ISRE-SEAP assay.

Method

[1256] The IFNα2 albumin fusion protein encoded by construct ID # 2249 can be tested for activity in the ISRE-SEAP assay as previously described in Example 76. Briefly, conditioned yeast supernatants were tested at a 1:1000 dilution for their ability to direct ISRE signal transduction on the ISRE-SEAP/293F reporter cell-line. The ISRE-SEAP/293F reporter cells were plated at 3×10^4 cell/well in 96-well, poly-D-lysine coated, plates, one day prior to treatment. The reporter cells were then incubated for 18 or 24 hours prior to removing 40 µL for use in the SEAP Reporter Gene Chemiluminescent Assay (Roche catalog # 1779842). Recombinant human Interferon beta, "rhIFNb" (Biogen), was used as a positive control.

Result

[1257] The purified preparation of IFNα2-HSA demonstrated a relatively linear increase in the ISRE-SEAP assay over concentrations ranging from 10^{-1} to 10^{1} ng/mL (see Figure 5) or 10^{-10} to 10^{-6} ng/mL (see Figure 6).

In vivo induction of OAS by interferon alpha fusion encoded by construct ID 2249.

Method

[1258] The OAS enzyme, 2'-5'- OligoAdenylate Synthetase, is activated at the transcriptional level by interferon in response to antiviral infection. The effect of interferon constructs can be measured by obtaining blood samples from treated monkeys and analyzing these samples for transcriptional activation of two OAS mRNA, p41 and p69. A volume of 0.5 mL of whole blood was obtained from 4 animals per group at 7 different time points, day 0, day 1, day 2, day 4, day 8, day 10, and day 14 per animal. The various groups include vehicle control, intravenous injection of 30 µg/kg HSA-IFN on day 1, subcutaneous injection of 30 µg/kg of HSA-IFN on day 1, subcutaneous injection of 300 µg/kg of HSA-IFN on day 1, and subcutaneous injection of 40 µg/kg of interferon alpha (Schering-Plough) as a positive control on days 1, 3, and 5. The levels of the p41 and the p69 mRNA transcripts were determined by real-time quantitative PCR (Taqman) using probes specific for p41-OAS and p69-OAS. OAS mRNA levels were quantitated relative to 18S ribosomal RNA endogenous control. The albumin fusion encoded by construct 2249 can be subjected to similar experimentation.

Results

[1259] A significant increase in mRNA transcript levels for both p41 and p69 OAS was observed in HSA-interferon treated monkeys in contrast to IFNα treated monkeys (see Figure 7 for p41 data). The effect lasted nearly 10 days.

EXAMPLE 79: Indications for IFNα2 Albumin Fusion Proteins.

[1260] IFN alpha albumin fusion protein (including, but not limited to, those encoded by constructs 2249, 2343, 2410, 2366, 2382, and 2381) can be used to treat, prevent, ameliorate, and/or detect multiple sclerosis. Other indications include, but are not limited to viral infections including Severe Acute Respiratory Syndrome (SARS) and other coronavirus infections; filoviruses, including but not limited to Ebola viruses and Marburg

virus; Arenaviruses, including but not limited to Pichende virus, Lassa virus, Junin virus, Machupo virus, Guanarito virus; and lymphocytic choriomeningitis virus (LCMV); Bunyaviruses, including but not limited to Punta toro virus, Crimean-Congo hemorrhagic fever virus, sandfly fever viruses, Rift Valley fever virus, La Crosse virus, and hantaviruses; Flaviviruses, including but not limited to Yellow Fever, Banzi virus, West Nile virus, Dengue viruses, Japanese Encephalitis virus, Tick-borne encephalitis, Omsk Hemorrhagic Fever, and Kyasanur Forest Disease virus; Togaviruses, including but not limited to Venezuelan, eastern, and western equine encephalitis viruses, Ross River virus, and Rubella virus; Orthopox viruses, including but not limited to Vaccinia, Cowpox, Smallpox, and Monkeypox; Herpesviruses; FluA/B; Respiratory Syncytial virus (RSV); parnflu; measles; rhinoviruses; adenoviruses; Semliki Forest virus; Viral Hemorrhagic fevers; Rhabdoviruses; Paramyxoviruses, including but not limited to Nipah virus and Hendra virus; and other viral agents identified by the U.S. Centers for Disease Control and Prevention as high-priority disease agents (*i.e.*, Category A, B, and C agents; see, *e.g.*, Moran, Emerg. Med. Clin. North. Am. 2002; 20(2):311-30 and Darling et al., Emerg. Med. Clin. North Am. 2002;20(2):273-309).

[1261] Preferably, the IFNα-albumin fusion protein or IFN hybrid fusion protein is administered in combination with a CCR5 antagonist, further in association with at least one of ribavirin, IL-2, IL-12, pentafuside alone or in combination with an anti-HIV drug therapy, e.g., HAART, for preparation of a medicament for the treatment of HIV-1 infections, HCV, or HIV-1 and HCV co-infections in treatment-naive as well as treatment-experienced adult and pediatric patients.

Example 8b: Construct ID # 3691, BNP-HSA, Generation.

[1262] Construct ID # 3691, pC4:SPCON.BNP1-32/HSA, comprises DNA encoding a BNP albumin fusion protein which has a consensus leader sequence, secretion, followed by the processed, active BNP peptide (amino acids 1-32) fused to the amino-terminus of the mature form of HSA in the mammalian expression vector pC4.

Cloning of BNP cDNA for construct 3691

[1263] The polynucleotide encoding BNP was PCR amplified using primers BNP-1 and BNP-2, described below, cut with *Bam* HI/*Cla* I, and ligated into *Bam* HI/*Cla* I cut pC4:HSA resulting in construct ID # 3691. Construct ID # 3691 encodes an albumin fusion protein containing a consensus leader sequence (SEQ ID No:111) and the processed, active form of BNP, followed by the mature HSA protein (see SEQ ID No:321 for construct 3691 in Table 2).

[1264] Two oligonucleotides suitable for PCR amplification of the polynucleotide encoding the active, processed form of BNP, BNP-1 and BNP-2, were synthesized:

BNP-1: 5'-GAGCGCGGATCCAAGCTTCCGCCATC*ATGTGGTGGCGCCTGTGGTGGCTGCTGCTGCTGCTGCTGCTGTG GCCCATGGTGTGGGCC*AGCCCCAAGCTGGTGCAAGG -3' (SEQ ID NO:463)

BNP-2: 5'-AGTCCCATCGATGAGCAACCTCACTCTTGTGTGCATCATGCCGCCTCAGCACTTTGC-3' (SEQ ID NO:464).

[1265] BNP-1 incorporates a *Bam* HI cloning site (underlined), polynucleotides encoding a consensus leader sequence (SEQ ID No:111) (italicized), and polynucleotides encoding the first seven amino acid sequence of BNP (bolded). In BNP-2, the underlined sequence is a *Cla* I site, and the polynucleotides that follow it contains the reverse complement of DNA encoding the last 6 amino acids of BNP (bolded) and the first 10 amino acids of the mature HSA protein. Using these two primers the BNP protein was PCR amplified. Annealing and extension temperatures and times must be empirically determined for each specific primer pair and template.

[1266] The PCR product was purified (for example, using Wizard PCR Preps DNA Purification System (Promega Corp)) and then digested with *Bam* HI and *Cla* I. After further purification of the *Bam* HI-*Cla* I fragment by gel electrophoresis, the product was cloned into *Bam* HI /*Cla* I digested pC4:HSA to produce construct ID # 3691. The expression construct was sequence verified.

Expression and Purification of Construct ID 3691.

Expression in 293F cells.

[1267] Construct ID # 3691, pC4:SPCON.BNP1-32/HSA, was transfected into 293F cells by methods known in the art (see Example 6).

Purification from 293F cell supernatant.

[1268] Two liters of supernatant were collected 3 days post-transfection. The recombinant protein was captured by 5 ml Blue Sepharose CL-6B column (Amersham Biosciences, Piscataway, NJ, USA) and eluted by 2 M NaCl. The material was bound to HiPrep 16/10 Phenyl FF (high sub) column and eluted by 20 mM MES, pH 6.7. BNP-HSA was further purified by hydroxyapatite column chromatography in sodium phosphate buffer gradient (0–20 mS/cm in 200 ml) at pH 6.8. The final product was exchanged into PBS pH 7.2 by a HiPrep 26/10 desalting column (Amersham Biosciences).

The activity of BNP-HSA can be assayed using an in vitro NPR-A/cGMP Assay.

[1269] Natriuretic peptide receptor-A (NPR-A) is the signaling receptor for BNP, and as such, is responsible for most of BNP's biological effects. BNP bioactivity is mediated by NPR-A guanylyl cyclase domain that converts GTP to cGMP upon activation. A convenient assay for BNP activity is to measure the BNP stimulation of a 293F cell line that stably over-expresses NPR-A. The cGMP production in the cells after exposure to BNP can be measured by cGMP ELISA.

Method of Screening NPR-A 293F stable clones.

[1270] The open reading frame of human NPR-A was constructed into pcDNA3.1 expression vector (Invitrogen). 293F cells were stably transfected with the plasmid DNA by Lipofectamine method and selected by 0.8 μg/ml G418. 293F/NPR-A stable clones were screened for best response to recombinant BNP.

Measurement of cGMP activation.

[1271] cGMP activation by BNP was carried out in 293F/NPR-A cells and measured by CatchPoint cyclic-GMP fluorescent assay kit (Molecular Devices, Sunnyvale, CA, USA). Briefly, 50,000 cells/well of 293F/NPR-A cells cultured in a 96-well plate were washed into 80 μl prestimulation buffer (Krebs-Ringer Bicarbonate Buffer with 10 mM glucose, pH 7.4, 15 mM sodium bicarbonate, and 0.75 mM 3-isobutyl-1-methylxanthine). BNP-HSA or recombinant BNP in 40 μl prestimulation buffer was added to the cells at 37°C for 10 min. The cells were lysed with 40 μl Lysis Buffer for 10 min with shaking. The amounts of cGMP in the lysates were quantitated as per the manufacturer's instruction.

Result

[1272] The dose-response relationship of BNP-HSA and recombinant BNP were determined (see Figure 8). The maximal activities of Construct ID # 3691 and recombinant BNP were similar (1.63 ± 0.016 vs. 1.80 ± 0.016 pm, respectively), with EC50 values of 28.4 ± 1.2, and 0.46 ± 1.1 nM, respectively.

BNP-HSA decreases blood pressure in vivo.

Method

[1273] BNP reduces blood pressure by direct vasodilation as well as by suppression of renin/angiotensin/endothelin/aldosterone systems. The ability of BNP-HSA to decrease arterial blood pressure was tested in three-month old male spontaneously hypertensive rats purchased from Taconic (Germantown, NY, USA). Spontaneously hypertensive rats are genetically hypertensive with onset of high blood pressure after three months of age. BNP-HSA or recombinant BNP was reconstituted in 0.3 cc PBS per rat. The drugs were delivered via tail vein injection. Systolic and diastolic blood pressures were recorded by cuff-tail method using XBP-1000 System (Kent Scientific, Torrington, CT, USA). For each blood pressure data point, 4-5 consecutive readings were taken and averaged. Mean arterial pressure (MAP) was calculated as 1/3 systolic pressure + 2/3 diastolic pressure. For dose-response determination, blood pressures were measured 20 h after pC4:SPCON.BNP1-32/HSA administration at doses of 0.5, 2, 6, and 18 nmol/kg.

Result

[1274] The typical systolic pressure of spontaneously hypertensive rats was 180–200 mmHg before dosing. A single bolus of 6 nmol/kg BNP-HSA delivered via tail vein intravenous injection lowered both systolic and diastolic pressure, which accounted for more than 30 mmHg mean arterial pressure (MAP) reduction. The lowered blood pressure was steady and continued for a day and then gradually returned to the baseline over several days (see Figure 9). In contrast, due to its instantaneous clearance, a single 6 nmol/kg bolus of recombinant BNP, produced only a very transient MAP decrease of about ~15 mmHg.

[1275] In addition, the dose-response 20 hours post injection of a bolus of BNP-HSA was determined in four spontaneously hypertensive rats. 0.5 nmol/kg BNP-HSA had an average of 7 mmHg MAP reduction, while 6 nmol/kg BNP-HSA had an average of 30 mmHg MAP reduction, and a high dose of 18 nmol/kg BNP-HSA only lowered the blood pressure slightly more than 6 nmol/kg.

In vivo induction of plasma cGMP by BNP-HSA.

Method

[1276] The intracellular cGMP activation by BNP results in its release from the cell to circulation. The plasma cGMP level correlates with BNP-induced cardiovascular and renal physiology. Plasma cGMP has been used as a biomarker for *in vivo* BNP action. To test the induction of plasma cGMP by BNP-HSA *in vivo*, eleven- to 12-week-old male C57/BL6 mice received a single bolus of recombinant BNP or BNP-HSA at a 6 nmol/kg dose via tail vein. Plasma was prepared from the tail bleeds at 5, 10, 20, 40, and 80 min time points for the recombinant BNP dosing group and at additional 640, 1440, 2880, and 5760 min for the BNP-HSA group. Plasma samples from mice treated with PBS as the vehicle control were collected as the zero time points. cGMP levels were determined by CatchPoint cyclic-GMP fluorescent assay kit according to the manufacture's instruction.

Result

[1277] Following a single intravenous bolus of 6 nmol/kg recombinant BNP or BNP-HSA, peak plasma cGMP levels over the baseline were increased 3.9- or 5.6-fold, respectively (see Figure 10). In addition, the one-phase exponential decay half-life of cGMP following recombinant BNP treatment was 16 min (10 to 42 min, 95%CI), while the one-phase exponential decay half-life of cGMP following BNP-HSA administration was 1538 min (1017 to 3153 min, 95%CI).

In vivo Pharmacokinetic analysis of BNP albumin fusion encoded by construct ID 3691.

Method

[1278] Eleven- to 12-week-old male C57/BL6 mice (obtained from Ace Animals, Boyertown, PA, USA) weighed 25.1 ± 0.12 g at the time of the study. All animals were dosed at a volume of 10 ml/kg body weight. Predose animals were injected with PBS. Recombinant BNP was injected intravenously in the tail or subcutaneously in the mid-scapular region.

[1279] Pharmacokinetic analysis was performed on the following groups:

Table 8.

Group	Drug	Dose (mg/kg)	Route	N/time	Time (hours)
1	BNP-HSA	2.19	Subcutaneous	3	0.5, 2, 6, 16, 24, 32, 48, 72, 96
2	BNP-HSA	2.19	Intravenous	3	0.083, 2, 6, 16, 24, 32, 48, 72, 96
3	Vehicle	0	Subcutaneous	3	predose
4	Vehicle	0	Intravenous	3	predose

[1280] Blood was sampled from the inferior vena cava, placed into an EDTA-coated microtainer, and stored on ice. The samples were centrifuged in a microcentrifuge at 14,000 rpm (16,000 ×g) for 10 minutes at room temperature. The plasma was transferred into cluster tubes and stored at –80°C.

[1281] BNP-HSA concentrations in plasma samples were determined using BNP EIA Kit (Phoenix Pharmaceutical, Belmont, CA, USA). The standard curves were generated at the same time on the same plate with testing samples. The detection limit was 0.11 ng/mL for recombinant BNP. The assay detects recombinant BNP and does not cross react to mouse BNP.

[1282] Analysis was conducted by noncompartmental methods (WinNonlin; version 4.1; Pharsight Corp.,Mountain View, CA, USA). The mean plasma concentration at each time was used in the analysis. A linear up/log down trapezoidal method was used to calculate the AUC_{0-t}. Extrapolation to infinity $AUC_{0-\infty}$ was done by dividing the last observed concentration by the terminal elimination rate constant. Data were uniformly weighted for these analyses.

Result

[1283] The mean baseline concentration of BNP-HSA in plasma as detected in the pre-dose samples was approximately 0.081–0.093 µg/ml. Following a single intravenous or subcutaneous injection, BNP-HSA had terminal elimination half-lives of 11.2 (intravenous delivery) or 19.3 h (subcutaneous delivery), while the half-life of recombinant BNP in mice was 3.1 min. Non compartmental analysis of BNP-HSA revealed that BNP-HSA had the following characteristics:

Table 9.

	Unit	Intravenous	Subcutaneous
t_{max}	h	NA	16
C_{max}	µg/ml	NA	11.2
$t_{1/2,term}$	h	11.2	19.3
$AUC_{0-\infty}$	(h · µg/ml)/(mg/kg)	658.9	227.9
V_{ss}	ml/kg	37	NA
V_z or V_z/F	ml/kg	53.5	268
CL or CL/F	ml/h/kg	3.3	9.6
MRT	h	11.2	19.8
Bioavailability	%		34.6
C_{max}, peak plasma concentration of the drug; t_{max}, time of maximum plasma concentration; $AUC_{0-\infty}$, area under the plasma drug concentration-time curve from time 0 to infinite time; $t_{1/2,term}$, terminal elimination phase half-life; CL, clearance after intravenous dosing; CL/F, apparent clearance after subcutaneous dosing; V_{ss}, volume of distribution at steady-state after intravenous dosing; V_z: volume of distribution during the terminal phase after intravenous dosing; V_z/F, volume of distribution during the terminal phase after subcutaneous dosing; NA, not applicable.			

[1284] Five points at the terminal phase of the intravenous profile and four points at the terminal phase of the subcutaneous profile were selected for the terminal half-life calculation. The resulting AUC during this terminal phase was approximately 10% of the total AUC for the intravenous and subcutaneous profiles, respectively. This is compared to only 2% and 4% of the total AUC for the intravenous and subcutaneous profile, respectively, when the last three points were selected for the terminal half-life calculation.

EXAMPLE 81: Construct ID # 3618, BNP(2X)-HSA, Generation.

[1285] Construct ID # 3618, pC4:SPCON.BNP1-32(2x)/HSA, comprises DNA encoding a BNP albumin fusion protein which has a consensus leader sequence, secreton, followed by two processed, active BNP peptides (amino acids 1-32) in tandem fused to the amino-terminus of the mature form of HSA in the mammalian expression vector pC4.

Cloning of BNP cDNA for construct 3618

[1286] The polynucleotide encoding the duplicate BNP moiety was first PCR amplified from the processed active form of BNP (amino acids 1-32) using four primers BNP-1, BNP-2, BNP-3, and BNP-4, described below, to create two fragments A and B. Following amplification, two purified fragments (A and B) were mixed in an equal molar amount and used as a PCR template and amplified with primers BNP-5 and BNP-6, as

described below. The BNP(2X) insert was then digested with *Bam*HI and *Cla*I and ligated into pC4HSA vector pre-digested with *Bam*HI and *Cla*I resulting in construct 3618. Construct ID # 3618 encodes an albumin fusion protein containing a consensus leader sequence, secrecon (SEQ ID No:111), and two, tandem copies of the processed, active form of BNP, followed by the mature HSA protein (see SEQ ID No:483 for construct 3618 in Table 2).

[1287] Four oligonucleotides suitable for PCR amplification of the polynucleotides encoding two fragments of BNP protein were first synthesized:

BNP-1 5'AGCCCCAAGATGGTGCAAGGGTCTGGCTGCTTTGGGAGGAAGATGGACCGGATCAGCTCCTCCACTG GCTGGGCT GCAAAGTGCTGAGGCGGCAT-3' (SEQ ID NO:486)

BNP-2 5'-CCTTGCACCATCTTGGGGCTATGCCGCCTCAGCACTTTGC-3' (SEQ ID NO:487)

BNP-3 5'-GCAAAGTGCTGAGGCGGCATAGCCCCAAGATGGTGCAAGG-3' (SEQ ID NO:488)

BNP-4 5'-AGTCCCATCGATGAGCAACCTCACTCTTGTGTGCATCATGCCGCCTC AGCACTTTGC-3' (SEQ ID NO:489)

[1288] Using primer sets BNP-1/BNP-2 and BNP-3/BNP-4, two BNP protein fragments (A and B, respectively) were PCR amplified. Annealing and extension temperatures and times must be empirically determined for each specific primer pair and template. Fragments A and B were purified (for example, using Wizard PCR Preps DNA Purification System (Promega Corp)), mixed at equal molar amounts, and used as a template for PCR amplification using two additional oligonucleotides suitable for PCR amplification, BNP-5 and BNP-6:

BNP-5: 5'-GAGCGCGGATCCAAGCTTCCGCCATC*ATGTGGTGGCGCCTGTGGTGGCTGCTGCTGCTGCTGCTGCTGCT GTGGCCCATGGTGTGGGCCAGCCCCAAGCTGGTGCAAGG* -3' (SEQ ID NO:463)

BNP-6: 5'-AGTCCCATCGATGAGCAACCTCACTCTTGTGTGCATCATGCCGCCTCAGCACTTTGC-3' (SEQ ID NO:464)

[1289] BNP-5 incorporates a *Bam* HI cloning site (underlined), polynucleotides encoding a consensus leader sequence (SEQ ID No:111) (italicized), and polynucleotides encoding the first seven amino acid sequence of BNP (bolded). In BNP-6, the underlined sequence is a *Cla* I site, and the polynucleotides that follow it contains the reverse complement of DNA encoding the last 6 amino acids of BNP (bolded) and the first 10 amino acids of the mature HSA protein. Using these two primers, a consensus leader sequence and two tandem copies of active BNP peptides were PCR amplified. Annealing and extension temperatures and times must be empirically determined for each specific primer pair and template.

[1290] The PCR product was purified (for example, using Wizard PCR Preps DNA Purification System (Promega Corp)) and then digested with *Bam* HI and *Cla* I. After further purification of the *Bam* HI-*Cla* I fragment by gel electrophoresis, the product was cloned into *Bam* HI /*Cla* I digested pC4:HSA to produce construct ID # 3618. The expression construct was sequence verified.

Expression and Purification of Construct ID # 3618.

Expression in 293F cells.

[1291] Construct ID # 3618, pC4:SPCON.BNP1-32(2x)/HSA, was transfected into 293F cells by methods known in the art (see Example 6).

Purification from 293F cell supernatant.

[1292] pC4:SPCON.BNP1-32(2x)/HSA encoded by Construct ID # 3618 was purified as previously described above in Example 80 under subsection heading "Purification from 293F cell supernatant."

The Activity of BNP(2X)-HSA can be assayed using an In Vitro NPR-A/cGMP Assay.

[1293] The activity of BNP(2X)-HSA encoded by Construct ID # 3618 can be assayed *in vitro* using an NPR-A/cGMP assay as previously described in Example 80 under subsection heading, "The activity of BNP-HSA can be assayed using an in vitro NPR-A/cGMP Assay," and "Method of Screening NPR-A 293F Stable Clones."

Result

[1294] The dose-response relationship of BNP(2X)-HSA and recombinant BNP were determined (see Figure 8). The maximal activities of BNP(2X)-HSA encoded by Construct ID # 3618, and recombinant BNP were similar (1.68 ± 0.021, vs. 1.80 ± 0.016 pm, respectively), with EC50 values of 9.8 ± 1.1, and 0.46 ± 1.1 nM respectively.

[1295] The entire disclosure of each document cited (including patents, patent applications, patent publications, journal articles, abstracts, laboratory manuals, books, or other disclosures) as well as information available through identifiers specific to databases such as GenBank, GeneSeq, or the CAS Registry, referred to in this application are herein incorporated by reference in their entirety.

[1296] Furthermore, the specification and sequence listing of each of the following U.S. applications are herein incorporated by reference in their entirety: U.S. Application No. 60/542,274, filed February 4, 2005; U.S. Application No. 60/549,901, filed March 5, 2004; U.S. Application No. 60/556,906, filed March 29, 2004; and U.S. Application No. 60/636,603, filed December 17, 2004.

Applicant's File Reference Number: PF612PCT	International Application Number: Unassigned

INDICATIONS RELATING TO DEPOSITED BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited biological material referred to in Table 3, page 25 of the description.

B. IDENTIFICATION OF DEPOSIT: Further deposits are identified on an additional sheet: ☐

Name of Depository: American Type Culture Collection
Address of Depository: 10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

	Accession Number	Date of Deposit		Accession Number	Date of Deposit
1	PTA-3759	04-Oct-2001	2	PTA-3764	04-Oct-2001
3	PTA-3941	19-Dec-2001	4	PTA-3763	04-Oct-2001
5	PTA-3940	19-Dec-2001	6	PTA-3942	19-Dec-2001
7	PTA-3939	19-Dec-2001	8	PTA-3943	19-Dec-2001
9	PTA-4670	16-Sep-2002	10		

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CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

What is claimed:

1. An albumin fusion protein comprising a member selected from the group consisting of:

(a) a Therapeutic protein:X and albumin comprising the amino acid sequence of SEQ ID NO:1;

(b) a Therapeutic protein:X and a fragment or a variant of the amino acid sequence of SEQ ID NO:1, wherein said fragment or variant has albumin activity;

(c) a Therapeutic protein:X and a fragment or a variant of the amino acid sequence of SEQ ID NO:1, wherein said fragment or variant has albumin activity, and further wherein said albumin activity is the ability to prolong the shelf life of the Therapeutic protein:X compared to the shelf-life of the Therapeutic protein:X in an unfused state;

(d) a Therapeutic protein:X and a fragment or a variant of the amino acid sequence of SEQ ID NO:1, wherein said fragment or variant has albumin activity, and further wherein the fragment or variant comprises the amino acid sequence of amino acids 1-387 of SEQ ID NO:1;

(e) a fragment or variant of a Therapeutic protein:X and albumin comprising the amino acid sequence of SEQ ID NO:1, wherein said fragment or variant has a biological activity of the Therapeutic protein:X;

(f) a Therapeutic protein:X, or fragment or variant thereof, and albumin, or fragment or variant thereof, of (a) to (e), wherein the Therapeutic protein:X, or fragment or variant thereof, is fused to the N-terminus of albumin, or the N-terminus of the fragment or variant of albumin;

(g) a Therapeutic protein:X, or fragment or variant thereof, and albumin, or fragment or variant thereof, of (a) to (e), wherein the Therapeutic protein:X, or fragment or variant thereof, is fused to the C-terminus of albumin, or the C-terminus of the fragment or variant of albumin;

(h) a Therapeutic protein:X, or fragment or variant thereof, and albumin, or fragment or variant thereof, of (a) to (e), wherein the Therapeutic protein:X, or fragment or variant thereof, is fused to the N- terminus and C-terminus of albumin, or the N-terminus and the C-terminus of the fragment or variant of albumin;

(i) a Therapeutic protein:X, or fragment or variant thereof, and albumin, or fragment or variant thereof, of (a) to (e), which comprises a first Therapeutic protein:X, or fragment or variant thereof, and a second Therapeutic protein:X, or fragment or variant thereof, wherein said first Therapeutic protein:X, or fragment or variant thereof, is different from said second Therapeutic protein:X, or fragment or variant thereof;

(j) a Therapeutic protein:X, or fragment or variant thereof, and albumin, or fragment or variant thereof, of (a) to (i), wherein the Therapeutic protein:X, or fragment or variant thereof, is separated from the albumin or the fragment or variant of albumin by a linker; and

(k) a Therapeutic protein:X, or fragment or variant thereof, and albumin, or fragment or variant thereof, of (a) to (j), wherein the albumin fusion protein has the following formula:

R1-L-R2; R2-L-R1; or R1-L-R2-L-R1,

and further wherein R1 is Therapeutic protein:X, or fragment or variant thereof, L is a peptide linker, and R2 is albumin comprising the amino acid sequence of SEQ ID NO:1 or a fragment or variant of albumin.

2. The albumin fusion protein of claim 1, wherein the shelf-life or plasma stability of the albumin fusion protein is greater than the shelf-life or plasma stability of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

3. The albumin fusion protein of claim 1, wherein the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin, or fragment or variant thereof, is greater than the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

4. The albumin fusion protein of claim 1, wherein the in vivo biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin, or fragment or variant thereof, is greater than the in vivo biological activity of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

5. An albumin fusion protein comprising a Therapeutic protein:X, or fragment or variant thereof, inserted into an albumin, or fragment or variant thereof, comprising the amino acid sequence of SEQ ID NO:1 or fragment or variant thereof.

6. An albumin fusion protein comprising a Therapeutic protein:X, or fragment or variant thereof, inserted into an albumin, or

fragment or variant thereof, comprising an amino acid sequence selected from the group consisting of:

(a) amino acids 54 to 61 of SEQ ID NO:1;
(b) amino acids 76 to 89 of SEQ ID NO:1;
(c) amino acids 92 to100 of SEQ ID NO:1;
(d) amino acids 170 to 176 of SEQ ID NO:1;
(e) amino acids 247 to 252 of SEQ ID NO:1;
(f) amino acids 266 to 277 of SEQ ID NO:1;
(g) amino acids 280 to 288 of SEQ ID NO:1;
(h) amino acids 362 to 368 of SEQ ID NO:1;
(i) amino acids 439 to 447 of SEQ ID NO:1;
(j) amino acids 462 to 475 of SEQ ID NO:1;
(k) amino acids 478 to 486 of SEQ ID NO:1; and
(l) amino acids 560 to 566 of SEQ ID NO:1.

7. The albumin fusion protein of claim 5, wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the shelf-life or plasma stability of the Therapeutic protein:X, or fragment or variant thereof, as compared to the shelf-life or plasma stability of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

8. The albumin fusion protein of claim 6, wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the shelf-life or plasma stability of the Therapeutic protein:X, or fragment or variant thereof, as compared to the shelf-life or plasma stability of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

9. The albumin fusion protein of claim 5, wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin as compared to the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

10. The albumin fusion protein of claim 6, wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin as compared to the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

11. The albumin fusion protein of claim 5 wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the in vivo biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin compared to the in vivo biological activity of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

12. The albumin fusion protein of claim 6 wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the in vivo biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin compared to the in vivo biological activity of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

13. The albumin fusion protein of any one of claims 1-12, which is non-glycosylated.

14. The albumin fusion protein of any one of claims 1-12, which is expressed in yeast.

15. The albumin fusion protein of claim 14, wherein the yeast is glycosylation deficient.

16. The albumin fusion protein of claim 14 wherein the yeast is glycosylation and protease deficient.

17. The albumin fusion protein of any one of claims 1-12, which is expressed by a mammalian cell.

18. The albumin fusion protein of any one of claims 1-12, wherein the albumin fusion protein is expressed by a mammalian cell in culture.

19. The albumm fusion protein of any one of claims 1-12, wherein the albumin fusion protein further comprises a secretion leader sequence.

20. A composition comprising the albumin fusion protein of any one of claims 1-12 and a pharmaceutically acceptable carrier.

21. A kit comprising the composition of claim 20.

22. A method of treating a disease or disorder in a patient, comprising the step of administering the albumin fusion protein of any one of claims 1-12.

23. The method of claim 22, wherein the disease or disorder comprises indication:Y.

24. A method of treating a patient with a disease or disorder that is modulated by Therapeutic protein:X, or fragment or variant thereof, comprising the step of administering an effective amount of the albumin fusion protein of any one of claims 1-12.

25. The method of claim 24, wherein the disease or disorder is indication:Y.

26. A method of extending the shelf life or plasma stability of Therapeutic protein:X, or fragment or variant thereof, comprising the step of fusing the Therapeutic protein:X, or fragment or variant thereof, to albumin, or fragment or variant thereof, sufficient to extend the shelf-life or plasma stability of the Therapeutic protein:X, or fragment or variant thereof, compared to the shelf-life of the Therapeutic protein:X, or fragment or variant thereof, in an unfused state.

27. A nucleic acid molecule comprising a polynucleotide sequence encoding the albumin fusion protein of any one of claims 1-12.

28. A vector comprising the nucleic acid molecule of claim 27.

29. A host cell comprising the nucleic acid molecule of claim 28.

```
  1 GAT GCA CAC AAG AGT GAG GTT GCT CAT CGG TTT AAA GAT TTG GGA GAA GAA AAT TTC AAA 60
  1 D   A   H   K   S   E   V   A   H   R   F   K   D   L   G   E   E   N   F   K   20

 61 GCC TTG GTG TTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120
 21 A   L   V   L   I   A   F   A   Q   Y   L   Q   Q   C   P   F   E   D   H   V   40

121 AAA TTA GTG AAT GAA GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GCT GAA 180
 41 K   L   V   N   E   V   T   E   F   A   K   T   C   V   A   D   E   S   A   E   60

181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240
 61 N   C   D   K   S   L   H   T   L   F   G   D   K   L   C   T   V   A   T   L   80

241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300
 81 R   E   T   Y   G   E   M   A   D   C   C   A   K   Q   E   P   E   R   N   E   100

301 TGC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 360
101 C   F   L   Q   H   K   D   D   N   P   N   L   P   R   L   V   R   P   E   V   120

361 GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420
121 D   V   M   C   T   A   F   H   D   N   E   E   T   F   L   K   K   Y   L   Y   140

421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480
141 E   I   A   R   R   H   P   Y   F   Y   A   P   E   L   L   F   F   A   K   R   160
```

Figure 1A

```
481 TAT AAA GCT GCT TTT ACA GAA TGT TGC CAA GCT GCT GAT AAA GCT GCC TGC CTG TTG CCA 540
161 Y   K   A   A   F   T   E   C   C   Q   A   A   D   K   A   A   C   L   L   P   180

541 AAG CTC GAT GAA CTT CGG GAT GAA GGG AAG GCT TCG TCT GCC AAA CAG AGA CTC AAA TGT 600
181 K   L   D   E   L   R   D   E   G   K   A   S   S   A   K   Q   R   L   K   C   200

601 GCC AGT CTC CAA AAA TTT GGA GAA AGA GCT TTC AAA GCA TGG GCA GTG GCT CGC CTG AGC 660
201 A   S   L   Q   K   F   G   E   R   A   F   K   A   W   A   V   A   R   L   S   220

661 CAG AGA TTT CCC AAA GCT GAG TTT GCA GAA GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA 720
221 Q   R   F   P   K   A   E   F   A   E   V   S   K   L   V   T   D   L   T   K   240

721 GTC CAC ACG GAA TGC TGC CAT GGA GAT CTG CTT GAA TGT GCT GAT GAC AGG GCG GAC CTT 780
241 V   H   T   E   C   C   H   G   D   L   L   E   C   A   D   D   R   A   D   L   260

781 GCC AAG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA 840
261 A   K   Y   I   C   E   N   Q   D   S   I   S   S   K   L   K   E   C   C   E   280

841 AAA CCT CTG TTG GAA AAA TCC CAC TGC ATT GCC GAA GTG GAA AAT GAT GAG ATG CCT GCT 900
281 K   P   L   L   E   K   S   H   C   I   A   E   V   E   N   D   E   M   P   A   300

901 GAC TTG CCT TCA TTA GCT GCT GAT TTT GTT GAA AGT AAG GAT GTT TGC AAA AAC TAT GCT 960
301 D   L   P   S   L   A   A   D   F   V   E   S   K   D   V   C   K   N   Y   A   320
```

Figure 1B

```
 961 GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TTG TAT GAA TAT GCA AGA AGG CAT CCT GAT 1020
 321 E   A   K   D   V   F   L   G   M   F   L   Y   E   Y   A   R   R   H   P   D   340

1021 TAC TCT GTC GTG CTG CTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080
 341 Y   S   V   V   L   L   L   R   L   A   K   T   Y   E   T   T   L   E   K   C   360

1081 TGT GCC GCT GCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CTT 1140
 361 C   A   A   A   D   P   H   E   C   Y   A   K   V   F   D   E   F   K   P   L   380

1141 GTG GAA GAG CCT CAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200
 381 V   E   E   P   Q   N   L   I   K   Q   N   C   E   L   F   E   Q   L   G   E   400

1201 TAC AAA TTC CAG AAT GCG CTA TTA GTT CGT TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT 1260
 401 Y   K   F   Q   N   A   L   L   V   R   Y   T   K   K   V   P   Q   V   S   T   420

1261 CCA ACT CTT GTA GAG GTC TCA AGA AAC CTA GGA AAA GTG GGC AGC AAA TGT TGT AAA CAT 1320
 421 P   T   L   V   E   V   S   R   N   L   G   K   V   G   S   K   C   C   K   H   440

1321 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTG AAC CAG TTA 1380
 441 P   E   A   K   R   M   P   C   A   E   D   Y   L   S   V   V   L   N   Q   L   460

1381 TGT GTG TTG CAT GAG AAA ACG CCA GTA AGT GAC AGA GTC ACA AAA TGC TGC ACA GAG TCC 1440
 461 C   V   L   H   E   K   T   P   V   S   D   R   V   T   K   C   C   T   E   S   480
```

Figure 1C

```
1441 TTG GTG AAC AGG CGA CCA TGC TTT TCA GCT CTG GAA GTC GAT GAA ACA TAC GTT CCC AAA 1500
 481 L   V   N   R   R   P   C   F   S   A   L   E   V   D   E   T   Y   V   P   K   500

1501 GAG TTT AAT GCT GAA ACA TTC ACC TTC CAT GCA GAT ATA TGC ACA CTT TCT GAG AAG GAG 1560
 501 E   F   N   A   E   T   F   T   F   H   A   D   I   C   T   L   S   E   K   E   520

1561 AGA CAA ATC AAG AAA CAA ACT GCA CTT GTT GAG CTT GTG AAA CAC AAG CCC AAG GCA ACA 1620
 521 R   Q   I   K   K   Q   T   A   L   V   E   L   V   K   H   K   P   K   A   T   540

1621 AAA GAG CAA CTG AAA GCT GTT ATG GAT GAT TTC GCA GCT TTT GTA GAG AAG TGC TGC AAG 1680
 541 K   E   Q   L   K   A   V   M   D   D   F   A   A   F   V   E   K   C   C   K   560

1681 GCT GAC GAT AAG GAG ACC TGC TTT GCC GAG GAG GGT AAA AAA CTT GTT GCT GCA AGT CAA 1740
 561 A   D   D   K   E   T   C   F   A   E   E   G   K   K   L   V   A   A   S   Q   580

1741 GCT GCC TTA GGC TTA TAA CAT CTA CAT TTA AAA GCA TCT CAG 1782
 581 A   A   L   G   L   *                                   585
```


Figure 1D

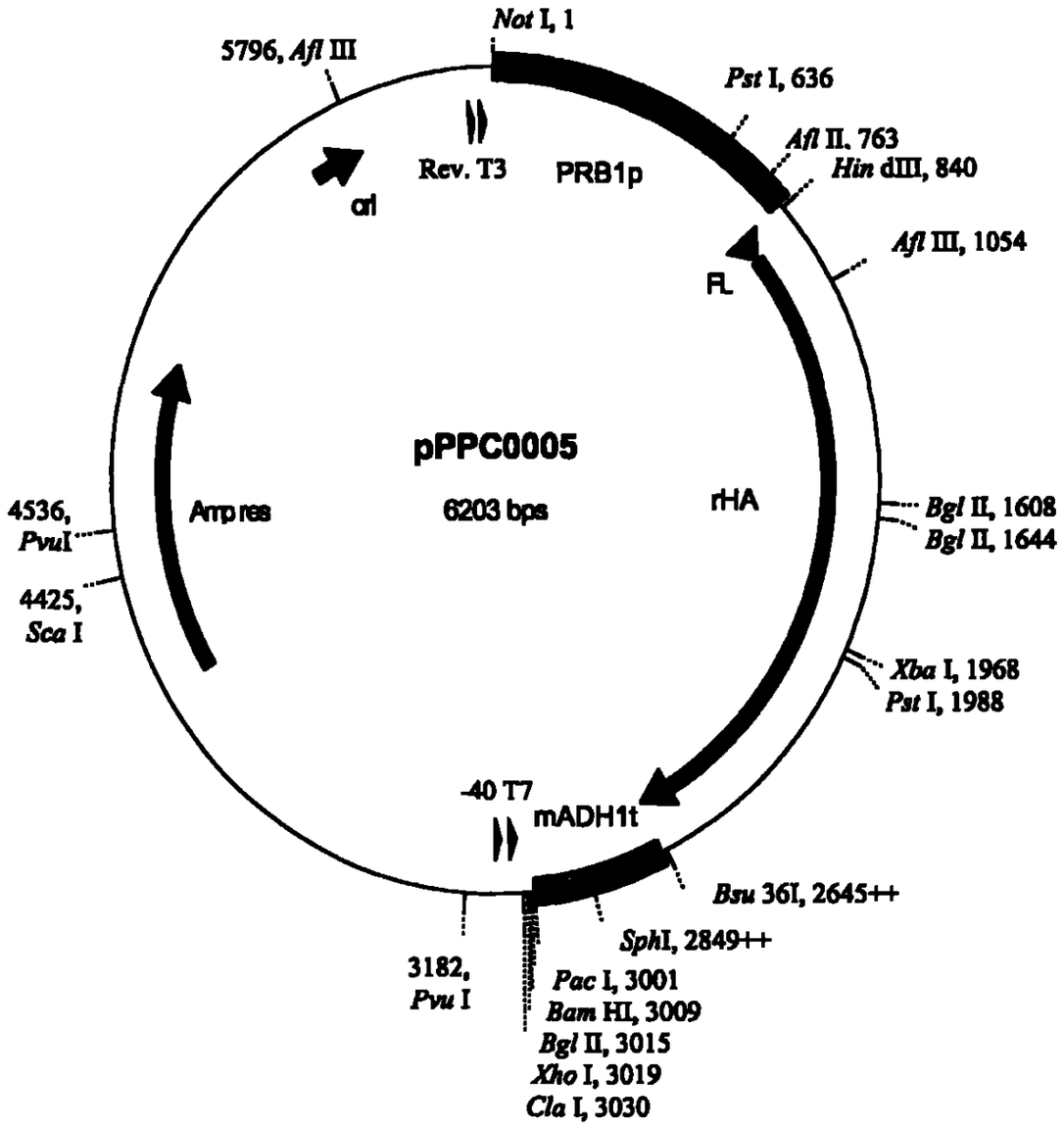

Not I, 1
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Hin dIII, 840
Afl III, 1054
Rev. T3
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ori
FL
pPPC0005
6203 bps
rHA
Amp res
4536,
PvuI
4425,
Sca I
Bgl II, 1608
Bgl II, 1644
Xba I, 1968
Pst I, 1988
-40 T7
mADH1t
Bsu 36I, 2645++
SphI, 2849++
3182,
Pvu I
Pac I, 3001
Bam HI, 3009
Bgl II, 3015
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Cla I, 3030

Figure 2

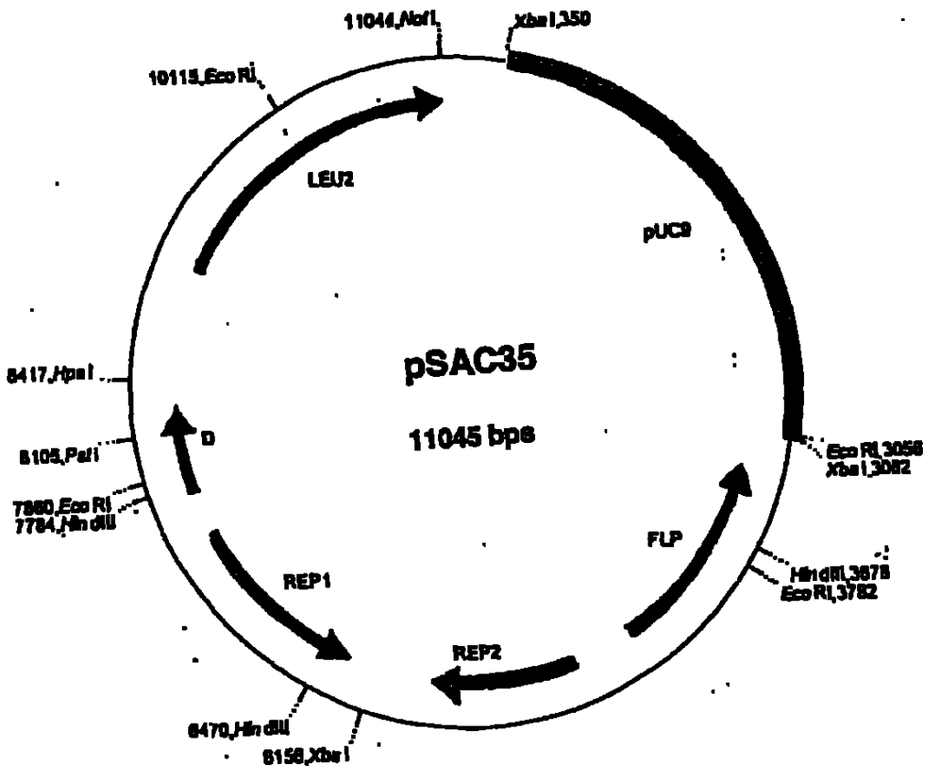

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XbaI,350
10115,EcoRI
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8417,HpaI
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7880,EcoRI
7784,HindIII
D
REP1
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REP2
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Figure 3

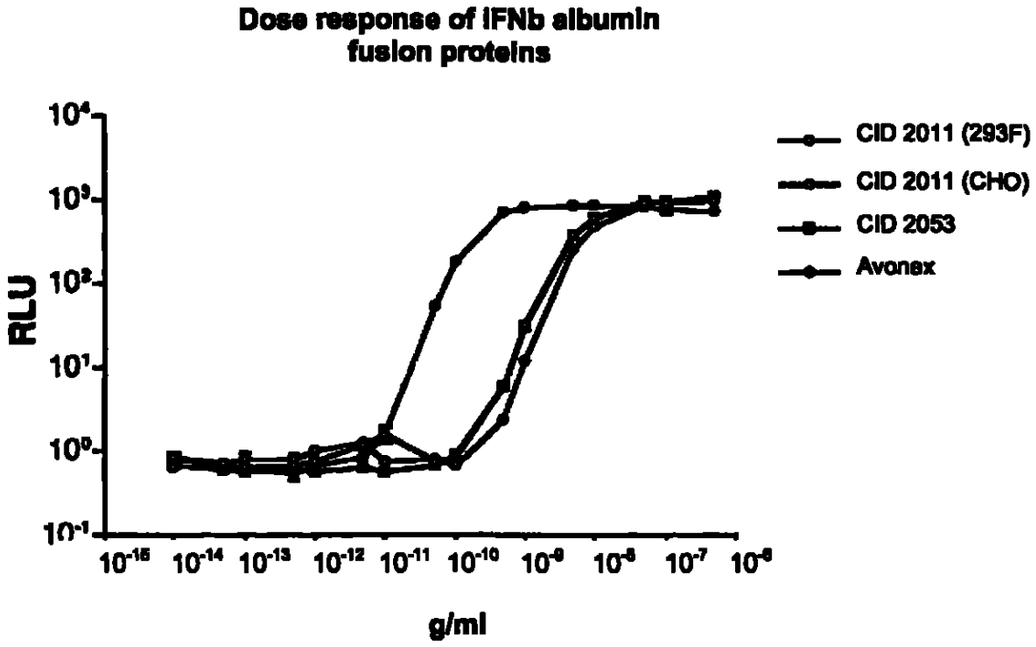

Dose response of IFNb albumin fusion proteins
RLU
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-15} 10^{-14} 10^{-13} 10^{-12} 10^{-11} 10^{-10} 10^{-9} 10^{-8} 10^{-7} 10^{-6}
g/ml
CID 2011 (293F)
CID 2011 (CHO)
CID 2053
Avonex

Figure 4

Inhibition of proliferation of HS294T melanoma cells by IFNa albumin fusion protein

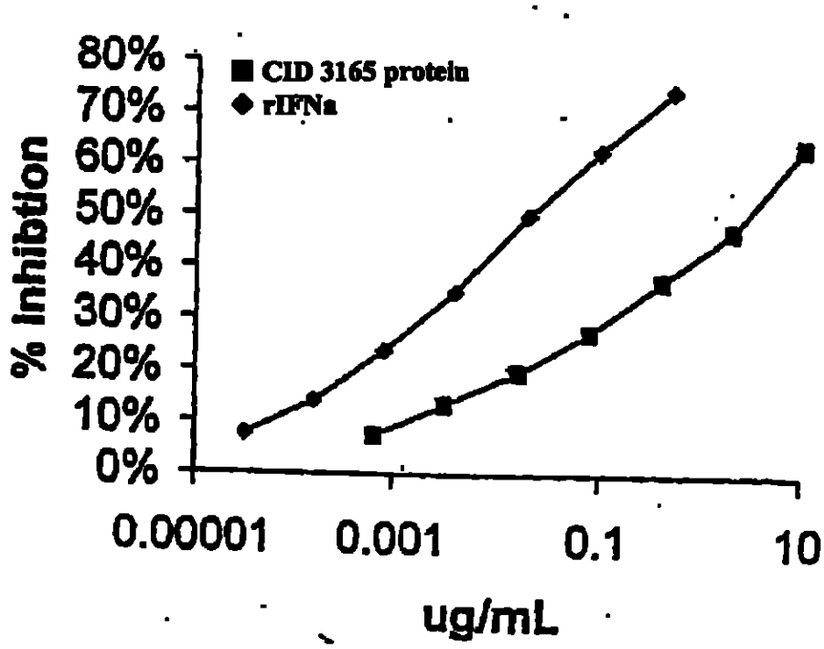


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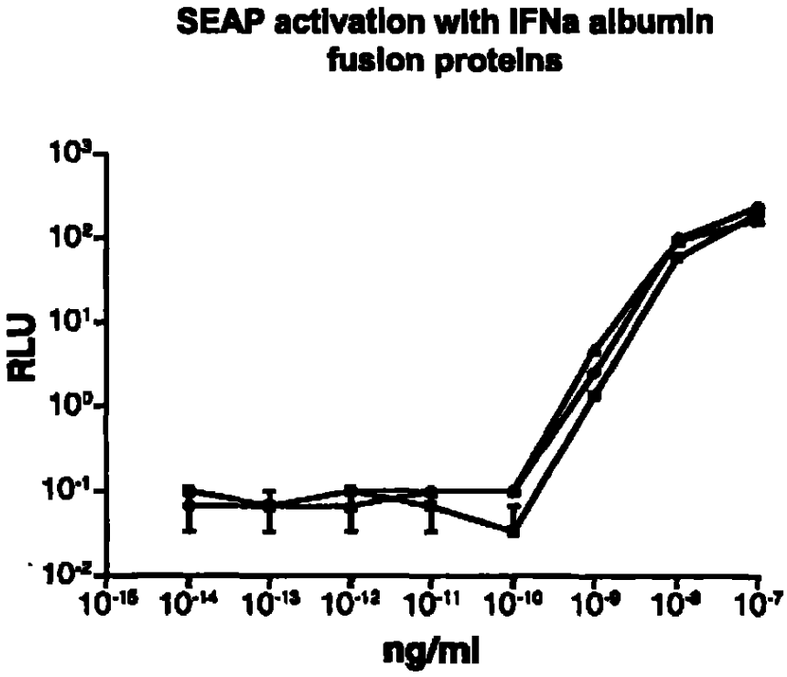

SEAP activation with IFNa albumin fusion proteins
RLU
10³
10²
10¹
10⁰
10⁻¹
10⁻²
10⁻¹⁵ 10⁻¹⁴ 10⁻¹³ 10⁻¹² 10⁻¹¹ 10⁻¹⁰ 10⁻⁹ 10⁻⁸ 10⁻⁷
ng/ml

Figure 6

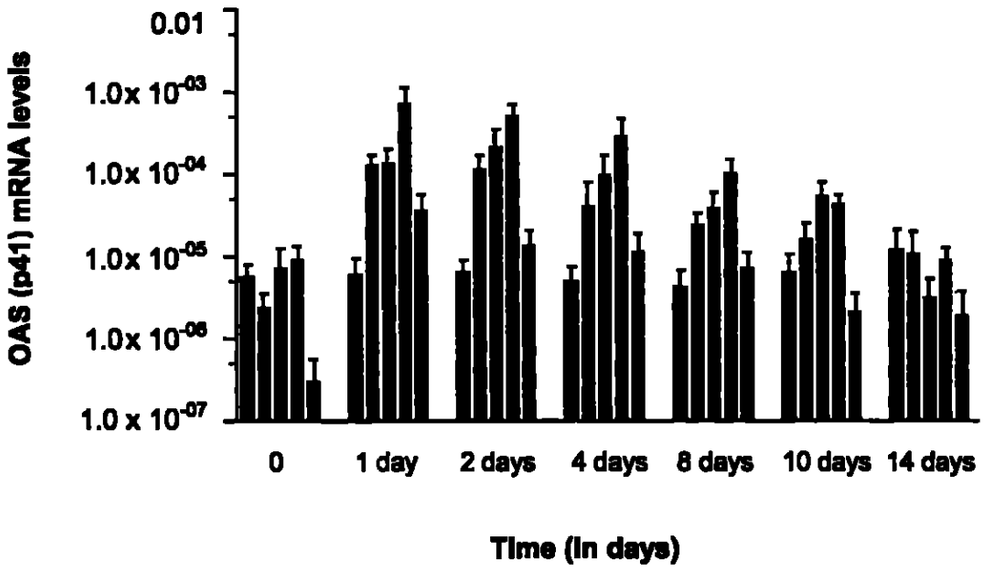

0.01
1.0x 10-03
1.0x 10-04
1.0x 10-05
1.0x 10-06
1.0 x 10-07
OAS (p41) mRNA levels
0
1 day
2 days
4 days
8 days
10 days
14 days
Time (in days)

Figure 7

Dose-response of recombinant BNP
and BNP Albumin Fusion Proteins —
BNP-HSA and BNP(2X)-HSA

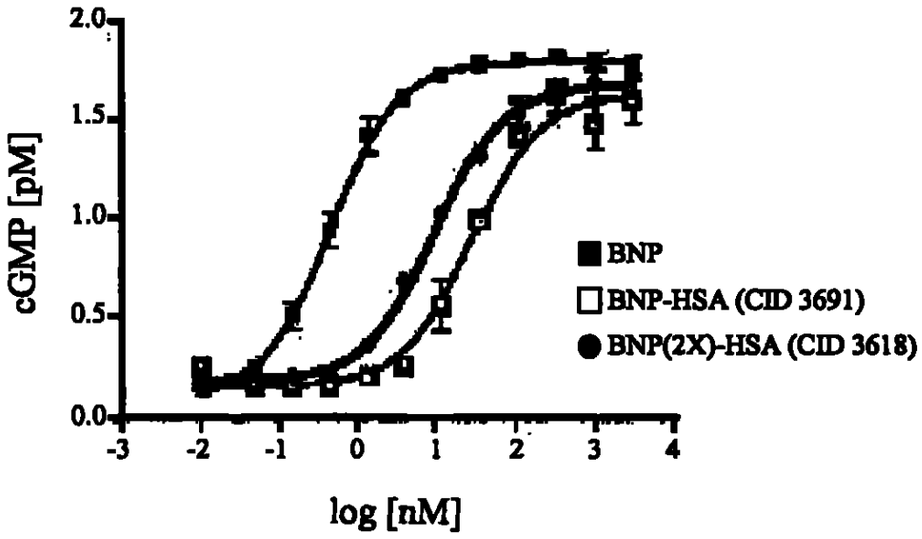


Figure 8

Effect of BNP Albumin Fusion Proteins
on Mean Arterial Pressure in
Spontaneously Hypertensive Rats

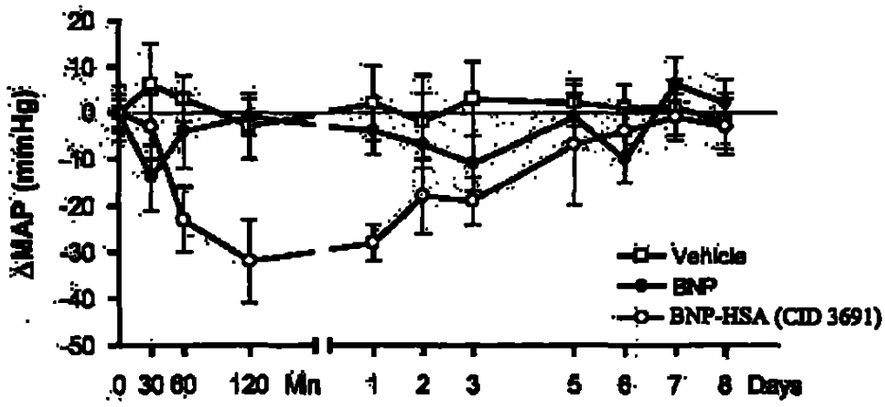


Figure 9

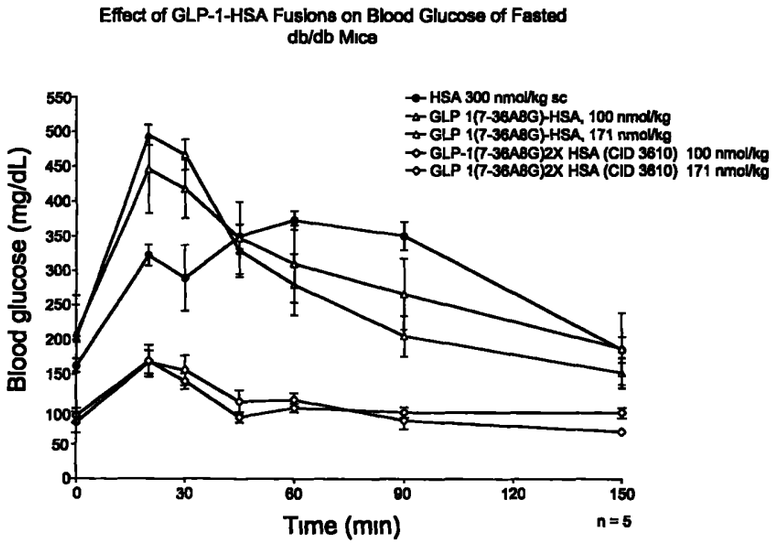


Figure 11

196

In Vivo cGMP Levels in Mice After an Intravenous Injection of Recombinant BNP or BNP Albumin Fusion Proteins

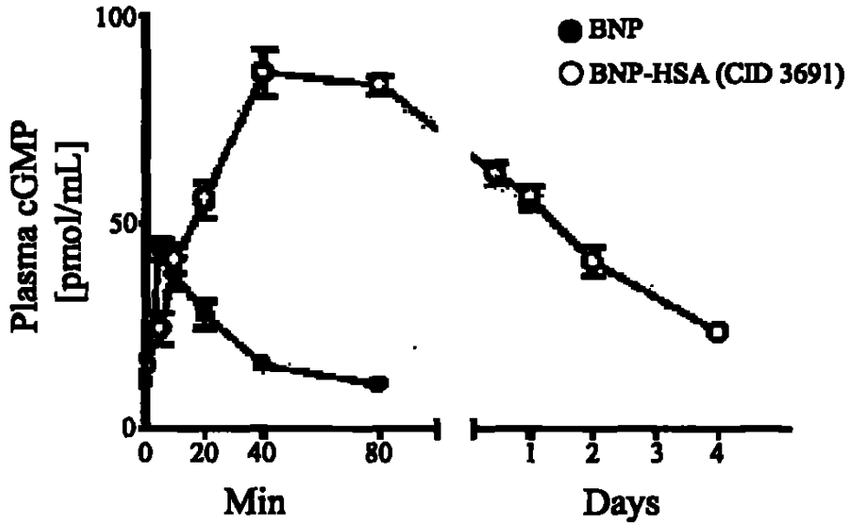


Figure 10

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Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
         35                  40                  45

Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
     50                  55                  60

Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
 65                  70                  75                  80

Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
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Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
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Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His
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    130                 135                 140

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Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala
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Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu
195 200 205

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210 215 220

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225 230 235 240

Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp
245 250 255

Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser
260 265 270

Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His
275 280 285

Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser
290 295 300

Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala
305 310 315 320

Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg
325 330 335

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340 345 350

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355 360 365

Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro
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485 490 495

Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp

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aaattagtga atgaagtaac tgaatttgca aaaacatgtg ttgctgatga gtcagctgaa     180
aattgtgaca aatcacttca tacccttttt ggagacaaat tatgcacagt tgcaactctt     240
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aaaataagat atatataagg ttaagtaaag cgtctgttag aaaggaagtt tttccttttt 300
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Phe Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu

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Ser Gly

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Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
1 5 10 15

Leu Glu His Thr His Arg Arg Gly Ser Leu Asp Lys Arg
20 25

<210> 33
<211> 23
<212> PRT
<213> Homo sapiens

<400> 33
Met Lys Leu Ala Tyr Ser Leu Leu Leu Pro Leu Ala Gly Val Ser Ala
1 5 10 15

Ser Val Ile Asn Tyr Lys Arg
20

<210> 34
<211> 65
<212> PRT
<213> Homo sapiens

<400> 34

Met Lys Leu Lys Thr Val Arg Ser Ala Val Leu Ser Ser Leu Phe Ala
1 5 10 15

Ser Gln Val Leu Gly Gln Pro Ile Asp Asp Thr Glu Ser Gln Thr Thr
20 25 30

Ser Val Asn Leu Met Ala Asp Asp Thr Glu Ser Ala Phe Ala Thr Gln
35 40 45

Thr Asn Ser Gly Gly Leu Asp Val Val Gly Leu Ile Ser Met Ala Lys
50 55 60

Arg
65

<210> 35
<211> 70
<212> PRT
<213> Homo sapiens

<400> 35
Met Lys Leu Lys Thr Val Arg Ser Ala Val Leu Ser Ser Leu Phe Ala
1 5 10 15

Ser Gln Val Leu Gly Gln Pro Ile Asp Asp Thr Glu Ser Gln Thr Thr
20 25 30

Ser Val Asn Leu Met Ala Asp Asp Thr Glu Ser Ala Phe Ala Thr Gln
35 40 45

Thr Asn Ser Gly Gly Leu Asp Val Val Gly Leu Ile Ser Met Ala Glu
50 55 60

Glu Gly Glu Pro Lys Arg
65 70

<210> 36
<211> 58
<212> DNA
<213> Artificial Sequence

<220>
<223> primer used to generate XhoI and ClaI
site in pPPC0006

<400> 36
gcctcgagaa aagagatgca cacaagagtg aggttgctca tcgatttaaa gatttggg 58

<210> 37
<211> 59
<212> DNA
<213> Artificial Sequence

<220>
<223> primer used in generation XhoI and ClaI
site in pPPC0006

<400> 37
aatcgatgag caacctcact cttgtgtgca tctcttttct cgaggctcct ggaataagc 59

<210> 38
<211> 24
<212> DNA
<213> Artificial Sequence

<220>

<223> primer used in generation XhoI and ClaI
site in pPPC0006
<400> 38
tacaaactta agagtccaat tagc 24

<210> 39
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> primer used in generation XhoI and ClaI
site in pPPC0006

<400> 39
cacttctcta gagtggtttc atatgtctt 29

<210> 40
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<221> Misc_Structure
<223> Synthetic oligonucleotide used to alter restriction
sites in pPPC0007

<400> 40
aagctgcctt aggcttataa taaggcgcgc cggccggccg tttaaactaa gcttaattct 60

<210> 41
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<221> Misc_Structure
<223> Synthetic oligonucleotide used to alter restriction
sites in pPPC0007

<400> 41
agaattaagc ttagtttaaa cggccggccg gcgcgcctta ttataagcct aaggcagctt 60

<210> 42
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer useful for generation of albumin fusion protein in which the albumin moiety is N-terminal of the Therapeutic Protein

<220>
<221> misc_feature
<222> (18)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (19)
<223> n equals a,t,g, or c

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<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (32)
<223> n equals a,t,g, or c

<400> 42
aagctgcctt aggcttannn nnnnnnnnnn nn 32

<210> 43
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> reverse primer useful for generation of albumin fusion protein in which the albumin moiety is N-terminal of the Therapeutic Protein

<220>
<221> misc_feature
<222> (37)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (38)
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<222> (45)
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<222> (46)
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<220>
<221> misc_feature
<222> (48)
<223> n equals a,t,g, or c

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<221> misc_feature
<222> (49)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (50)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (51)
<223> n equals a,t,g, or c

<400> 43
gcgcgcgttt aaacggccgg ccggcgcgcc ttattannnn nnnnnnnnnn n 51

<210> 44
<211> 4
<212> PRT
<213> Homo sapiens

<400> 44
Leu Asp Lys Arg
1

<210> 45
<211> 4

<212> PRT
<213> Homo sapiens

<400> 45
Leu Glu Lys Arg
1

<210> 46
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer useful for generation of albumin fusion protein in which the albumin moiety is c-terminal of the Therapeutic Protein

<220>
<221> misc_feature
<222> (19)
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<220>
<221> misc_feature
<222> (20)
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<222> (21)
<223> n equals a,t,g, or c

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<222> (22)
<223> n equals a,t,g, or c

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<223> n equals a,t,g, or c

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<221> misc_feature
<222> (24)
<223> n equals a,t,g, or c

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<221> misc_feature
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<223> n equals a,t,g, or c

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<221> misc_feature
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<223> n equals a,t,g, or c

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<221> misc_feature
<222> (27)
<223> n equals a,t,g, or c

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<221> misc_feature
<222> (28)
<223> n equals a,t,g, or c

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<221> misc_feature
<222> (29)
<223> n equals a,t,g, or c

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<221> misc_feature
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<220>
<221> misc_feature
<222> (31)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (32)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (33)
<223> n equals a,t,g, or c

<400> 46
aggagcgtcg acaaaagann nnnnnnnnnn nnn 33

<210> 47
<211> 52
<212> DNA
<213> Artificial Sequence

<220>
<223> reverse primer useful for generation of albumin fusion protein in which the albumin moiety is c-terminal of the Therapeutic Protein

<220>
<221> misc_feature
<222> (38)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (39)
<223> n equals a,t,g, or c

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<221> misc_feature
<222> (40)
<223> n equals a,t,g, or c

<220>

<221> misc_feature
<222> (41)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (42)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (43)
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<221> misc_feature
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<220>
<221> misc_feature
<222> (46)
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<221> misc_feature
<222> (47)
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<221> misc_feature
<222> (48)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (49)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (50)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (51)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (52)
<223> n equals a,t,g, or c

<400> 47
ctttaaatcg atgagcaacc tcactcttgt gtgcatcnnn nnnnnnnnnn nn 52

<210> 48
<211> 9
<212> PRT
<213> Homo sapiens

<400> 48
Asp Ala His Lys Ser Glu Val Ala His
1 5

<210> 49
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<222> (1) to (11)
<223> Kozak sequence

<400> 49
ccgccaccat g 11

<210> 50
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer useful for inserting Therapeutic protein into pC4:HSA vector

<220>
<221> misc_feature
<222> (29)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (30)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (31)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (32)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (33)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (34)

<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (35)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (36)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (37)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (38)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (39)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (40)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (41)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (42)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (43)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (44)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (45)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (46)
<223> n equals a,t,g, or c

<400> 50
ccgccgctcg aggggtgtgt ttcgtcgann nnnnnnnnnn nnnnnn 46

<210> 51
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> reverse primer useful for inserting Therapeutic protein into pC4:HSA vector

<220>
<221> misc_feature
<222> (38)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (39)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (40)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (41)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (42)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (43)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (44)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (45)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (46)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (47)

<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (48)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (49)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (50)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (51)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (52)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (53)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (54)
<223> n equals a,t,g, or c

<220>
<221> misc_feature
<222> (55)
<223> n equals a,t,g, or c

<400> 51
agtcccatcg atgagcaacc tcactcttgt gtgcatcnnn nnnnnnnnnn nnnnn 55

<210> 52
<211> 733
<212> DNA
<213> Homo sapiens

<400> 52
gggatccgga gcccaaatct tctgacaaaa ctcacacatg cccaccgtgc ccagcacctg 60
aattcgaggg tgcaccgtca gtcttcctct tccccccaaa acccaaggac accctcatga 120
tctcccggac cctgaggtc acatgcgtgg tggtggacgt aagccacgaa gaccctgagg 180
tcaagttcaa ctggtacgtg gacggcgtgg aggtgcataa tgccaagaca aagccgcggg 240
aggagcagta caacagcacg taccgtgtgg tcagcgtcct caccgtcctg caccaggact 300
ggctgaatgg caaggagtac aagtgcaagg tctccaacaa agccctccca acccccatcg 360
agaaaaccat ctccaaagcc aaagggcagc cccgagaacc acaggtgtac accctgcccc 420
catcccggga tgagctgacc aagaaccagg tcagcctgac ctgcctggtc aaaggcttct 480
atccaagcga catcgccgtg gagtgggaga gcaatgggca gccggagaac aactacaaga 540

ccacgcctcc cgtgctggac tccgacggct ccttcttcct ctacagcaag ctcaccgtgg 600
acaagagcag gtggcagcag gggaacgtct tctcatgctc cgtgatgcat gaggctctgc 660
acaaccacta cacgcagaag agcctctccc tgtctccggg taaatgagtg cgacggccgc 720
gactctagag gat 733

<210> 53
<211> 5
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<222> (3)
<223> Xaa equals any of the twenty naturally ocurring L-amino acids

<400> 53
Trp Ser Xaa Trp Ser
1 5

<210> 54
<211> 86
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic sequence with 4 tandem copies of the GAS binding site found in the IRF1 promoter (Rothman et al., Immunity 1:457-468 (1994)), 18 nucleotides complementary to the SV40 early promoter, and a Xho I restriction site.

<400> 54
gcgcctcgag atttccccga aatctagatt tccccgaaat gatttccccg aaatgatttc 60
cccgaaatat ctgccatctc aattag 86

<210> 55
<211> 27
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic sequence complementary to the SV40 promter; includes a Hind III restriction site.

<400> 55
gcggcaagct ttttgcaaag cctaggc 27

<210> 56
<211> 271
<212> DNA
<213> Artificial Sequence

<220>
<221> Protein_Bind
<223> Synthetic promoter for use in biological assays; includes GAS binding sites found in the IRF1 promoter

<400> 56
ctcgagattt ccccgaaatc tagatttccc cgaaatgatt tccccgaaat gatttccccg 60
aaatatctgc catctcaatt agtcagcaac catagtcccg cccctaactc cgcccatccc 120

223

gcccctaact ccgcccagtt ccgcccattc tccgccccat ggctgactaa ttttttttat 180
ttatgcagag gccgaggccg cctcggcctc tgagctattc cagaagtagt gaggaggctt 240
ttttggaggc ctaggctttt gcaaaaagct t 271

<210> 57
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic primer complementary to human genomic EGR-1 promoter sequence; includes a Xho I restriction site.

<400> 57
gcgctcgagg gatgacagcg atagaacccc gg 32

<210> 58
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic primer complementary to human genomic EGR-1 promoter sequence; includes a Hind III restriction site.

<400> 58
gcgaagcttc gcgactcccc ggatccgcct c 31

<210> 59
<211> 12
<212> DNA
<213> Homo sapiens

<400> 59
ggggactttc cc 12

<210> 60
<211> 73
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic primer with 4 tandem copies of the NF-KB binding site (GGGGACTTTCCC), 18 nucleotides complementary to the 5' end of the SV40 early promoter sequence, and a XhoI restriction site.

<400> 60
gcggcctcga ggggactttc ccggggactt tccggggact ttccatcctg 60
ccatctcaat tag 73

<210> 61
<211> 256
<212> DNA
<213> Artificial Sequence

<220>
<221> Protein_Bind
<223> Synthetic promoter for use in biological assays; includes NF-KB binding sites.

<400> 61

ctcgagggga ctttcccggg gactttccgg ggactttccg ggactttcca tctgccatct 60
caattagtca gcaaccatag tcccgccccc aactccgccc atcccgcccc taactccgcc 120
cagttccgcc cattctccgc cccatggctg actaattttt tttatttatg cagaggccga 180
ggccgcctcg gcctctgagc tattccagaa gtagtgagga ggcttttttg gaggcctagg 240
cttttgcaaa aagctt 256

<210> 62
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate VH forward primer useful for amplifying human VH domains

<400> 62
caggtgcagc tggtgcagtc tgg 23

<210> 63
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate VH forward primer useful for amplifying human VH domains

<400> 63
caggtcaact taagggagtc tgg 23

<210> 64
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate VH forward primer useful for amplifying human VH domains

<400> 64
gaggtgcagc tggtggagtc tgg 23

<210> 65
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate VH forward primer useful for amplifying human VH domains

<400> 65
caggtgcagc tgcaggagtc ggg 23

<210> 66
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate VH forward primer useful for amplifying human VH domains

<400> 66
gaggtgcagc tgttgcagtc tgc 23

<210> 67
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate VH forward primer useful for amplifying human VH domains

<400> 67
caggtacagc tgcagcagtc agg 23

<210> 68
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate JH reverse primer useful for amplifying human VH domains

<400> 68
tgaggagacg gtgaccaggg tgcc 24

<210> 69
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate JH reverse primer useful for amplifying human VH domains

<400> 69
tgaagagacg gtgaccattg tccc 24

<210> 70
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate JH reverse primer useful for amplifying human VH domains

<400> 70
tgaggagacg gtgaccaggg ttcc 24

<210> 71
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate JH reverse primer useful for amplifying human VH domains

<400> 71
tgaggagacg gtgaccgtgg tccc 24

<210> 72
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Vkappa forward primer useful for amplifying human VL domains

<400> 72
gacatccaga tgacccagtc tcc 23

<210> 73
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Vkappa forward primer useful for amplifying human VL domains

<400> 73
gatgttgtga tgactcagtc tcc 23

<210> 74
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Vkappa forward primer useful for amplifying human VL domains

<400> 74
gatattgtga tgactcagtc tcc 23

<210> 75
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Vkappa forward primer useful for amplifying human VL domains

<400> 75
gaaattgtgt tgacgcagtc tcc 23

<210> 76
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Vkappa forward primer useful for amplifying human VL domains

<400> 76
gacatcgtga tgacccagtc tcc 23

<210> 77
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Vkappa forward primer useful for amplifying human VL domains

<400> 77
gaaacgacac tcacgcagtc tcc 23

<210> 78
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Vkappa forward primer useful for amplifying human VL domains

<400> 78
gaaattgtgc tgactcagtc tcc 23

<210> 79
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Vlambda forward primer useful for amplifying human VL domains

<400> 79
cagtctgtgt tgacgcagcc gcc 23

<210> 80
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Vlambda forward primer useful for amplifying human VL domains

<400> 80
cagtctgccc tgactcagcc tgc 23

<210> 81
<211> 23
<212> DNA
<213> Artificial Sequence

<220>

<223> Degenerate Vlambda forward primer useful for amplifying human VL domains

<400> 81
tcctatgtgc tgactcagcc acc 23

<210> 82
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Vlambda forward primer useful for amplifying human VL domains

<400> 82
tcttctgagc tgactcagga ccc 23

<210> 83
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Vlambda forward primer useful for amplifying human VL domains

<400> 83
cacgttatac tgactcaacc gcc 23

<210> 84
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Vlambda forward primer useful for amplifying human VL domains

<400> 84
caggctgtgc tcactcagcc gtc 23

<210> 85
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Vlambda forward primer useful for amplifying human VL domains

<400> 85
aattttatgc tgactcagcc cca 23

<210> 86
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Jkappa reverse primer useful for

amplifying human VL domains

<400> 86
acgtttgatt tccaccttgg tccc 24

<210> 87
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Jkappa reverse primer useful for amplifying human VL domains

<400> 87
acgtttgatc tccagcttgg tccc 24

<210> 88
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Jkappa reverse primer useful for amplifying human VL domains

<400> 88
acgtttgata tccactttgg tccc 24

<210> 89
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Jkappa reverse primer useful for amplifying human VL domains

<400> 89
acgtttgatc tccaccttgg tccc 24

<210> 90
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Jkappa reverse primer useful for amplifying human VL domains

<400> 90
acgtttaatc tccagtcgtg tccc 24

<210> 91
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Jlambda reverse primer useful for amplifying human VL domains

<400> 91
cagtctgtgt tgacgcagcc gcc 23

<210> 92
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Jlambda reverse primer useful for amplifying human VL domains

<400> 92
cagtctgccc tgactcagcc tgc 23

<210> 93
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Jlambda reverse primer useful for amplifying human VL domains

<400> 93
tcctatgtgc tgactcagcc acc 23

<210> 94
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Jlambda reverse primer useful for amplifying human VL domains

<400> 94
tcttctgagc tgactcagga ccc 23

<210> 95
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Jlambda reverse primer useful for amplifying human VL domains

<400> 95
cacgttatac tgactcaacc gcc 23

<210> 96
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Jlambda reverse primer useful for amplifying human VL domains

<400> 96
caggctgtgc tcactcagcc gtc 23

<210> 97
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate Jlambda reverse primer useful for amplifying human VL domains

<400> 97
aattttatgc tgactcagcc cca 23

<210> 98
<211> 5
<212> PRT
<213> Homo sapiens

<400> 98
Cys Asp Leu Pro Gln
1 5

<210> 99
<211> 165
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<222> (23)
<223> Xaa equals Arg or Lys

<220>
<221> MISC_FEATURE
<222> (113)
<223> Xaa equals Ala or Val

<400> 99
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Xaa Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Thr Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Asp Leu
65 70 75 80

Leu Asp Lys Phe Cys Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Met Gln Glu Glu Arg Val Gly Glu Thr Pro Leu Met Asn
100 105 110

Xaa Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Arg Arg Ile Thr Leu
115 120 125

Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Leu Ser Leu Ser Thr Asn Leu Gln Glu Arg
145 150 155 160

Leu Arg Arg Lys Glu
165

<210> 100
<211> 165
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<222> (23)
<223> Xaa equals Arg or Lys

<220>
<221> MISC_FEATURE
<222> (113)
<223> Xaa equals Ala or Val

<400> 100
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Xaa Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Met Gln Glu Glu Arg Val Gly Glu Thr Pro Leu Met Asn
100 105 110

Xaa Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Arg Arg Ile Thr Leu
115 120 125

Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Leu Ser Leu Ser Thr Asn Leu Gln Glu Arg
145 150 155 160

Leu Arg Arg Lys Glu
165

<210> 101
<211> 165
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<222> (23)
<223> Xaa equals Arg or Lys

<400> 101
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Xaa Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Met Glu
85 90 95

Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu Met Asn
100 105 110

Val Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Lys Ile Phe Gln Glu Arg
145 150 155 160

Leu Arg Arg Lys Glu
165

<210> 102
<211> 165
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<222> (23)
<223> Xaa equals Arg or Lys

<220>
<221> MISC_FEATURE
<222> (97)
<223> Xaa equals Ser or Val

<220>

<221> MISC_FEATURE
<222> (98)
<223> Xaa equals Cys or Leu

<220>
<221> MISC_FEATURE
<222> (99)
<223> Xaa equals Val or Cys

<220>
<221> MISC_FEATURE
<222> (100)
<223> Xaa equals Met or Asp

<400> 102
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Xaa Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Xaa Xaa Xaa Xaa Gln Glu Val Gly Val Ile Glu Ser Pro Leu Met Tyr
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Thr Glu Lys Lys Tyr Ser Ser Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Ile Asn Leu Gln Lys Arg
145 150 155 160

Leu Lys Ser Lys Glu
165

<210> 103
<211> 167
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<222> (115)
<223> Xaa equals Ala or Val

<400> 103
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
1 5 10 15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
20 25 30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
35 40 45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
50 55 60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ala Ala Trp Asp Glu
65 70 75 80

Asp Leu Leu Asp Lys Phe Cys Thr Glu Leu Tyr Gln Gln Leu Asn Asp
85 90 95

Leu Glu Ala Cys Val Met Gln Glu Glu Arg Val Gly Glu Thr Pro Leu
100 105 110

Met Asn Xaa Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Arg Arg Ile
115 120 125

Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val
130 135 140

Val Arg Ala Glu Ile Met Arg Ser Leu Ser Leu Ser Thr Asn Leu Gln
145 150 155 160

Glu Arg Leu Arg Arg Lys Glu
165

<210> 104
<211> 166
<212> PRT
<213> Homo sapiens

<400> 104
Met Cys Asp Leu Pro Glu Thr His Ser Leu Asp Asn Arg Arg Thr Leu
1 5 10 15

Met Leu Leu Ala Gln Met Ser Arg Ile Ser Pro Ser Ser Cys Leu Met
20 25 30

Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln
35 40 45

Phe Gln Lys Ala Pro Ala Ile Ser Val Leu His Glu Leu Ile Gln Gln
50 55 60

Ile Phe Asn Leu Phe Thr Thr Lys Asp Ser Ser Ser Thr Gly Trp Asn
65 70 75 80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
85 90 95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
100 105 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
115 120 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
130 135 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145 150 155 160

Thr Gly Tyr Leu Arg Asn
165

<210> 105
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic signal peptide

<400> 105
Met Arg Pro Thr Trp Ala Trp Trp Leu Phe Leu Val Leu Leu Leu Ala Leu
1 5 10 15

Trp Ala Pro Ala Arg Gly
20

<210> 106
<211> 0
<212> DNA
<213> Artificial sequence

<220>
<223> primer (deleted)

<400> 106
000

<210> 107
<211> 106
<212> DNA
<213> Artificial sequence

<220>
<223> primer for full-length IFNb amplification, has BamHI cloning site

<400> 107
gcgcggatcc gaattccgcc gccatgacca acaagtgtct cctccaaatt gctctcctgt 60
tgtgcttctc cactacagct ctttccatga gctacaactt gcttgg 106

<210> 108
<211> 55
<212> DNA
<213> Artificial sequence

<220>
<223> primer for full-length IFNb amplification, has ClaI cloning site

<400> 108
gcgcgcatcg atgagcaacc tcactcttgt gtgcatcgtt tcggaggtaa cctgt 55

<210> 109

<211> 41
<212> DNA
<213> Homo sapiens

<400> 109
cgcgcgcgtc gacaaaagat gtgatctgcc tcaaacccac a 41

<210> 110
<211> 59
<212> DNA
<213> Homo sapiens

<400> 110
gcgcgcatcg atgagcaacc tcactcttgt gtgcatcttc cttacttctt aaactttct 59

<210> 111
<211> 21
<212> PRT
<213> Homo sapiens

<400> 111
Met Trp Trp Arg Leu Trp Trp Leu Leu Leu Leu Leu Leu Leu Leu Trp
1 5 10 15

Pro Met Val Trp Ala
20

<210> 112
<211> 24
<212> PRT
<213> Homo sapiens

<400> 112
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Gly Ser Leu Asp Lys Arg
20

<210> 113
<211> 1497
<212> DNA
<213> Hypocrea jecorina

<400> 113
tctagagttg tgaagtcggt aatcccgctg tatagtaata cgagtcgcat ctaaatactc 60
cgaagctgct gcgaacccgg agaatcgaga tgtgctggaa agcttctagc gagcggctaa 120
attagcatga aaggctatga gaaattctgg agacggcttg ttgaatcatg gcgttccatt 180
cttcgacaag caaagcgttc cgtcgcagta gcaggcactc attcccgaaa aaactcggag 240
attcctaagt agcgatggaa ccggaataat ataataggca atacattgag ttgcctcgac 300
ggttgcaatg caggggtact gagcttggac ataactgttc cgtaccccac ctcttctcaa 360
cctttggcgt ttccctgatt cagcgtaccc gtacaagtcg taatcactat taacccagac 420
tgaccggacg tgttttgccc ttcatttgga gaaataatgt cattgcgatg tgtaatttgc 480
ctgcttgacc gactggggct gttcgaagcc cgaatgtagg attgttatcc gaactctgct 540
cgtagaggca tgttgtgaat ctgtgtcggg caggacacgc ctcgaaggtt cacggcaagg 600
gaaaccaccg atagcagtgt ctagtagcaa cctgtaaagc cgcaatgcag catcactgga 660
aaatacaaac caatggctaa aagtacataa gttaatgcct aaagaagtca tataccagcg 720
gctaataatt gtacaatcaa gtggctaaac gtaccgtaat ttgccaacgg cttgtggggt 780
tgcagaagca acggcaaagc cccacttccc cacgtttgtt tcttcactca gtccaatctc 840

agctggtgat cccccaattg ggtcgcttgt ttgttccggt gaagtgaaag aagacagagg 900
taagaatgtc tgactcggag cgttttgcat acaaccaagg gcagtgatgg aagacagtga 960
aatgttgaca ttcaaggagt atttagccag ggatgcttga gtgtatcgtg taaggaggtt 1020
tgtctgccga tacgacgaat actgtatagt cacttctggt gaagtggtcc atattgaaat 1080
gtaagtcggc actgaacagg caaaagattg agttgaaact gcctaagatc tcgggccctc 1140
gggccttcgg cctttgggtg tacatgtttg tgctccgggc aaatgcaaag tgtggtagga 1200
tcgaacacac tgctgccttt accaagcagc tgagggtatg tgataggcaa atgttcaggg 1260
gccactgcat ggtttcgaat agaaagagaa gcttagccaa gaacaatagc cgataaagat 1320
agcctcatta aacggaatga gctagtaggc aaagtcagcg aatgtgtata tataaaggtt 1380
cgaggtccgt gcctccctca tgctctcccc atctactcat caactcagat cctccaggag 1440
acttgtacac catcttttga ggcacagaaa cccaatagtc aaccgcggac tggcatc 1497

<210> 114
<211> 366
<212> DNA
<213> Aspergillus nidulans

<400> 114
agatctggtt cctgagtaca tctaccgatg cgcctcgatc cccctcttag ccgcatgaga 60
ttcctaccat ttatgtccta tcgttcaggg tcctatttgg accgctagaa atagactctg 120
ctcgatttgt ttccattatt cacgcaatta cgatagtatt tggctctttt cgtttggccc 180
aggtcaattc gggtaagacg cgatcacgcc attgtggccg ccggcgttgt gctgctgcta 240
ttccccgcat ataaacaacc cctccaccag ttcgttgggc tttgcgaatg ctgtactcta 300
tttcaagttg tcaaaagaga ggattcaaaa aattataccc cagatatcaa agatatcaaa 360
gccatc 366

<210> 115
<211> 1646
<212> DNA
<213> Hypocrea jecorina

<400> 115
tctagagttg tgaagtcggt aatcccgctg tatagtaata cgagtcgcat ctaaatactc 60
cgaagctgct gcgaacccgg agaatcgaga tgtgctggaa agcttctagc gagcggctaa 120
attagcatga aaggctatga gaaattctgg agacggcttg ttgaatcatg gcgttccatt 180
cttcgacaag caaagcgttc cgtcgcagta gcaggcactc attcccgaaa aaactcggag 240
attcctaagt agcgatggaa ccggaataat ataataggca atacattgag ttgcctcgac 300
ggttgcaatg caggggtact gagcttggac ataactgttc cgtaccccac ctcttctcaa 360
cctttggcgt ttccctgatt cagcgtaccc gtacaagtcg taatcactat taacccagac 420
tgaccggacg tgttttgccc ttcatttgga gaaataatgt cattgcgatg tgtaatttgc 480
ctgcttgacc gactggggct gttcgaagcc cgaatgtagg attgttatcc gaactctgct 540
cgtagaggca tgttgtgaat ctgtgtcggg caggacacgc ctcgaaggtt cacggcaagg 600
gaaaccaccg atagcagtgt ctagtagcaa cctgtaaagc cgcaatgcag catcactgga 660
aaatacaaac caatggctaa aagtacataa gttaatgcct aaagaagtca tataccagcg 720
gctaataatt gtacaatcaa gtggctaaac gtaccgtaat ttgccaacgg cttgtggggt 780
tgcagaagca acggcaaagc cccacttccc cacgtttgtt tcttcactca gtccaatctc 840
agctggtgat cccccaattg ggtcgcttgt ttgttccggt gaagtgaaag aagacagagg 900
taagaatgtc tgactcggag cgttttgcat acaaccaagg gcagtgatgg aagacagtga 960
aatgttgaca ttcaaggagt atttagccag ggatgcttga gtgtatcgtg taaggaggtt 1020
tgtctgccga tacgacgaat actgtatagt cacttctggt gaagtggtcc atattgaaat 1080
gtaagtcggc actgaacagg caaaagattg agttgaaact gcctaagatc tcgggccctc 1140
gggccttcgg cctttgggtg tacatgtttg tgctccgggc aaatgcaaag tgtggtagga 1200
tcgaacacac tgctgccttt accaagcagc tgagggtatg tgataggcaa atgttcaggg 1260
gccactgcat ggtttcgaat agaaagagaa gcttagcctg cagcctctta tcgagaaaga 1320
aattaccgtc gctcgtgatt tgtttgcaaa aagaacaaaa ctgaaaaaac ccagacacgc 1380
tcgacttcct gtcttcctat tgattgcagc ttccaatttc gtcacacaac aaggtcctag 1440
cttagccaag aacaatagcc gataaagata gcctcattaa acggaatgag ctagtaggca 1500
aagtcagcga atgtgtatat ataaaggttc gaggtccgtg cctccctcat gctctcccca 1560
tctactcatc aactcagatc ctccaggaga cttgtacacc atcttttgag gcacagaaac 1620

ccaatagtca accgcggact ggcatc 1646

<210> 116
<211> 516
<212> DNA
<213> Aspergillus nidulans

<400> 116
agatctggtt cctgagtaca tctaccgatg cgcctcgatc cccctcttag ccgcatgaga 60
ttcctaccat ttatgtccta tcgttcaggg tcctatttgg accgctagaa atagactctg 120
ctcgatttgt ttccattatt cacgcaatta cgatagtatt tggctctttt cgtttggccc 180
aggtcaattc gggtaagacg cgatcacgcc attgtggccg ccggcgctgc agcctcttat 240
cgagaaagaa attaccgtcg ctcgtgattt gtttgcaaaa agaacaaaac tgaaaaaacc 300
cagacacgct cgacttcctg tcttcctatt gattgcagct tccaatttcg tcacacaaca 360
aggtcctacg ccggcgttgt gctgctgcta ttccccgcat ataaacaacc cctccaccag 420
ttcgttgggc tttgcgaatg ctgtactcta tttcaagttg tcaaaagaga ggattcaaaa 480
aattataccc cagatatcaa agatatcaaa gccatc 516

<210> 117
<211> 495
<212> DNA
<213> Homo sapiens

<400> 117
tgtgatctgc ctcaaaccca cagcctgggt tctagaagga ccttgatgct cctggcacag 60
atgaggagaa tctctctttt ctcctgcttg aaggacagac atgactttgg atttccccag 120
gaggagtttg gcaaccagtt ccaaaaggct gaaaccatcc ctgtcctcca tgagatgatc 180
cagcagatct tcaatctctt cagcacaaag gactcatctg ctgcttggga tgagaccctc 240
ctagacaaat tctacactga actctaccag cagctgaatg acctggaagc ctgtgtgata 300
caggggtgg gggtgacaga gactcccctg atgaaggagg actccattct ggctgtgagg 360
aaatacttcc aaagaatcac tctctatctg aaagagaaga aatacagccc ttgtgcctgg 420
gaggttgtca gagcagaaat catgagatct ttttctttgt caacaaactt gcaagaaagt 480
ttaagaagta aggaa 495

<210> 118
<211> 495
<212> DNA
<213> Homo sapiens

<400> 118
tgtgatctgc ctcaaaccca cagcctgggt tctagaagga ccttgatgct cctggcacag 60
atgaggagaa tctctctttt ctcctgcttg aaggacagac atgactttgg atttccccag 120
gaggagtttg gcaaccagtt ccaaaaggct gaaaccatcc ctgtcctcca tgagatgatc 180
cagcagatct tcaatctctt cagcacaaag gactcatctg ctgcttggga tgagaccctc 240
ctagacaaat tctacactga actctaccag cagctgaatg acctggaagc ctgtgtgata 300
caggggtgg gggtgacaga gactcccctg atgaaggagg actccattct ggctgtgagg 360
aaatacttcc aaagaatcac tctctatctg aaagagaaga aatacagccc ttgtgcctgg 420
gaggttgtca gagcagaaat catgagatct ttttctttgt caacaaactt gcaagaaagt 480
ttaagaagta aggaa 495

<210> 119
<211> 495
<212> DNA
<213> Homo sapiens

<400> 119
tgtgatctgc ctcaaaccca cagcctgggt tctagaagga ccttgatgct cctggcacag 60
atgaggagaa tctctctttt ctcctgcttg aaggacagac atgactttgg atttccccag 120
gaggagtttg gcaaccagtt ccaaaaggct gaaaccatcc ctgtcctcca tgagatgatc 180

cagcagatct tcaatctctt cagcacaaag gactcatctg ctgcttggga tgagaccctc 240
ctagacaaat tctacactga actctaccag cagctgaatg acctggaagc ctgtgtgata 300
caggggtgg gggtgacaga gactcccctg atgaaggagg actccattct ggctgtgagg 360
aaatacttcc aaagaatcac tctctatctg aaagagaaga aatacagccc ttgtgcctgg 420
gaggttgtca gagcagaaat catgagatct ttttctttgt caacaaactt gcaagaaagt 480
ttaagaagta aggaa 495

<210> 120
<211> 498
<212> DNA
<213> Homo sapiens

<400> 120
tgtgatctgc ctcaaaccca cagcctgggt tctagaagga ccttgatgct cctggcacag 60
atgaggagaa tctctctttt ctcctgcttg aaggacagac atgactttgg atttccccag 120
gaggagtttg gcaaccagtt ccaaaaggct gaaaccatcc ctgtcctcca tgagatgatc 180
cagcagatct tcaatctctt cagcacaaag gactcatctg ctgcttggga tgagaccctc 240
ctagacaaat tctacactga actctaccag cagctgaatg acctggaagc ctgtgtgata 300
caggggtgg gggtgacaga gactcccctg atgaaggagg actccattct ggctgtgagg 360
aaatacttcc aaagaatcac tctctatctg aaagagaaga aatacagccc ttgtgcctgg 420
gaggttgtca gagcagaaat catgagatct ttttctttgt caacaaactt gcaagaaagt 480
ttaagaagta aggaataa 498

<210> 121
<211> 495
<212> DNA
<213> Homo sapiens

<400> 121
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gaggagtttg gcaaccagtt ccaaaaggct gaaaccatcc ctgtcctcca tgagatgatc 180
cagcagatct tcaatctctt cagcacaaag gactcatctg ctgcttggga tgagaccctc 240
ctagacaaat tctacactga actctaccag cagctgaatg acctggaagc ctgtgtgata 300
caggggtgg gggtgacaga gactcccctg atgaaggagg actccattct ggctgtgagg 360
aaatacttcc aaagaatcac tctctatctg aaagagaaga aatacagccc ttgtgcctgg 420
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ttaagaagta aggaa 495

<210> 122
<211> 495
<212> DNA
<213> Homo sapiens

<400> 122
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gaggagtttg gcaaccagtt ccaaaaggct gaaaccatcc ctgtcctcca tgagatgatc 180
cagcagatct tcaatctctt cagcacaaag gactcatctg ctgcttggga tgagaccctc 240
ctagacaaat tctacactga actctaccag cagctgaatg acctggaagc ctgtgtgata 300
caggggtgg gggtgacaga gactcccctg atgaaggagg actccattct ggctgtgagg 360
aaatacttcc aaagaatcac tctctatctg aaagagaaga aatacagccc ttgtgcctgg 420
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ttaagaagta aggaa 495

<210> 123
<211> 495

<212> DNA
<213> Homo sapiens

<400> 123
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gaggagtttg gcaaccagtt ccaaaaggct gaaaccatcc ctgtcctcca tgagatgatc 180
cagcagatct tcaatctctt cagcacaaag gactcatctg ctgcttggga tgagaccctc 240
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gaggttgtca gagcagaaat catgagatct ttttctttgt caacaaactt gcaagaaagt 480
ttaagaagta aggaa 495

<210> 124
<211> 495
<212> DNA
<213> Homo sapiens

<400> 124
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atgaggagaa tctctctttt ctcctgcttg aaggacagac atgactttgg atttccccag 120
gaggagtttg gcaaccagtt ccaaaaggct gaaaccatcc ctgtcctcca tgagatgatc 180
cagcagatct tcaatctctt cagcacaaag gactcatctg ctgcttggga tgagaccctc 240
ctagacaaat tctacactga actctaccag cagctgaatg acctggaagc ctgtgtgata 300
caggggggtgg gggtgacaga gactcccctg atgaaggagg actccattct ggctgtgagg 360
aaatacttcc aaagaatcac tctctatctg aaagagaaga aatacagccc ttgtgcctgg 420
gaggttgtca gagcagaaat catgagatct ttttctttgt caacaaactt gcaagaaagt 480
ttaagaagta aggaa 495

<210> 125
<211> 495
<212> DNA
<213> Homo sapiens

<400> 125
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gaggagtttg gcaaccagtt ccaaaaggct gaaaccatcc ctgtcctcca tgagatgatc 180
cagcagatct tcaatctctt cagcacaaag gactcatctg ctgcttggga tgagaccctc 240
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gaggttgtca gagcagaaat catgagatct ttttctttgt caacaaactt gcaagaaagt 480
ttaagaagta aggaa 495

<210> 126
<211> 495
<212> DNA
<213> Homo sapiens

<400> 126
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atgaggagaa tctctctttt ctcctgcttg aaggacagac atgactttgg atttccccag 120
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cagcagatct tcaatctctt cagcacaaag gactcatctg ctgcttggga tgagaccctc 240
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caggggggtgg gggtgacaga gactcccctg atgaaggagg actccattct ggctgtgagg 360

aaatacttcc aaagaatcac tctctatctg aaagagaaga aatacagccc ttgtgcctgg 420
gaggttgtca gagcagaaat catgagatct ttttctttgt caacaaactt gcaagaaagt 480
ttaagaagta aggaa 495

<210> 127
<211> 501
<212> DNA
<213> Homo sapiens

<400> 127
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cctgaggaga ttaagcagct gcagcagttc cagaaggagg acgccgcatt gaccatctat 180
gagatgctcc agaacatctt tgctatttte agacaagatt catctagcac tggctggaat 240
gagactattg ttgagaacct cctggctaat gtctatcatc agataaacca tctgaagaca 300
gtcctggaag aaaaactgga gaaagaagat ttcaccaggg gaaaactcat gagcagtctg 360
cacctgaaaa gatattatgg gaggattctg cattacctga aggccaagga gtacagtcac 420
tgtgcctgga ccatagtcag agtggaaatc ctaaggaact tttacttcat taacagactt 480
acaggttacc tccgaaacta a 501

<210> 128
<211> 501
<212> DNA
<213> Homo sapiens

<400> 128
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ctgtggcaat tgaatgggag gcttgaatat tgcctcaagg acaggatgaa ctttgacatc 120
cctgaggaga ttaagcagct gcagcagttc cagaaggagg acgccgcatt gaccatctat 180
gagatgctcc agaacatctt tgctatttte agacaagatt catctagcac tggctggaat 240
gagactattg ttgagaacct cctggctaat gtctatcatc agataaacca tctgaagaca 300
gtcctggaag aaaaactgga gaaagaagat ttcaccaggg gaaaactcat gagcagtctg 360
cacctgaaaa gatattatgg gaggattctg cattacctga aggccaagga gtacagtcac 420
tgtgcctgga ccatagtcag agtggaaatc ctaaggaact tttacttcat taacagactt 480
acaggttacc tccgaaacta a 501

<210> 129
<211> 561
<212> DNA
<213> Homo sapiens

<400> 129
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tccatgagct acaacttgct tggattccta caaagaagca gcaattttca gtgtcagaag 120
ctcctgtggc aattgaatgg gaggcttgaa tattgcctca aggacaggat gaactttgac 180
atccctgagg agattaagca gctgcagcag ttccagaagg aggacgccgc attgaccatc 240
tatgagatgc tccagaacat ctttgctatt ttcagacaag attcatctag cactggctgg 300
aatgagacta ttgttgagaa cctcctggct aatgtctatc atcagataaa ccatctgaag 360
acagtcctgg aagaaaaact ggagaaagaa gatttcacca ggggaaaact catgagcagt 420
ctgcacctga aaagatatta tgggaggatt ctgcattacc tgaaggccaa ggagtacagt 480
cactgtgcct ggaccatagt cagagtggaa atcctaagga acttttactt cattaacaga 540
cttacaggtt acctccgaaa c 561

<210> 130
<211> 498
<212> DNA
<213> Homo sapiens

<400> 130
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ctgtggcaat tgaatgggag gcttgaatat tgcctcaagg acaggatgaa ctttgacatc 120
cctgaggaga ttaagcagct gcagcagttc cagaaggagg acgccgcatt gaccatctat 180
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gagactattg ttgagaacct cctggctaat gtctatcatc agataaacca tctgaagaca 300
gtcctggaag aaaaactgga gaaagaagat ttcaccaggg gaaaactcat gagcagtctg 360
cacctgaaaa gatattatgg gaggattctg cattacctga aggccaagga gtacagtcac 420
tgtgcctgga ccatagtcag agtggaaatc ctaaggaact tttacttcat taacagactt 480
acaggttacc tccgaaac 498

<210> 131
<211> 561
<212> DNA
<213> Homo sapiens

<400> 131
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tccatgagct acaacttgct tggattccta caaagaagca gcaattttca gtgtcagaag 120
ctcctgtggc aattgaatgg gaggcttgaa tattgcctca aggacaggat gaactttgac 180
atccctgagg agattaagca gctgcagcag ttccagaagg aggacgccgc attgaccatc 240
tatgagatgc tccagaacat ctttgctatt ttcagacaag attcatctag cactggctgg 300
aatgagacta ttgttgagaa cctcctggct aatgtctatc atcagataaa ccatctgaag 360
acagtcctgg aagaaaaact ggagaaagaa gatttcacca ggggaaaact catgagcagt 420
ctgcacctga aaagatatta tgggaggatt ctgcattacc tgaaggccaa ggagtacagt 480
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<210> 132
<211> 501
<212> DNA
<213> Homo sapiens

<400> 132
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ctgtggcaat tgaatgggag gcttgaatat tgcctcaagg acaggatgaa ctttgacatc 120
cctgaggaga ttaagcagct gcagcagttc cagaaggagg acgccgcatt gaccatctat 180
gagatgctcc agaacatctt tgctattttc agacaagatt catctagcac tggctggaat 240
gagactattg ttgagaacct cctggctaat gtctatcatc agataaacca tctgaagaca 300
gtcctggaag aaaaactgga gaaagaagat ttcaccaggg gaaaactcat gagcagtctg 360
cacctgaaaa gatattatgg gaggattctg cattacctga aggccaagga gtacagtcac 420
tgtgcctgga ccatagtcag agtggaaatc ctaaggaact tttacttcat taacagactt 480
acaggttacc tccgaaacta a 501

<210> 133
<211> 561
<212> DNA
<213> Homo sapiens

<400> 133
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tccatgagct acaacttgct tggattccta caaagaagca gcaattttca gtgtcagaag 120
ctcctgtggc aattgaatgg gaggcttgaa tattgcctca aggacaggat gaactttgac 180
atccctgagg agattaagca gctgcagcag ttccagaagg aggacgccgc attgaccatc 240
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aatgagacta ttgttgagaa cctcctggct aatgtctatc atcagataaa ccatctgaag 360
acagtcctgg aagaaaaact ggagaaagaa gatttcacca ggggaaaact catgagcagt 420
ctgcacctga aaagatatta tgggaggatt ctgcattacc tgaaggccaa ggagtacagt 480

cactgtgcct ggaccatagt cagagtggaa atcctaagga acttttactt cattaacaga 540
cttacaggtt acctccgaaa c 561

<210> 134
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<213> Homo sapiens

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tccatgagct acaacttgct tggattccta caaagaagca gcaattttca gtgtcagaag 120
ctcctgtggc aattgaatgg gaggcttgaa tattgcctca aggacaggat gaactttgac 180
atcctgagg agattaagca gctgcagcag ttccagaagg aggacgccgc attgaccatc 240
tatgagatgc tccagaacat ctttgctatt ttcagacaag attcatctag cactggctgg 300
aatgagacta ttgttgagaa cctcctggct aatgtctatc atcagataaa ccatctgaag 360
acagtcctgg aagaaaaact ggagaaagaa gatttcacca ggggaaaact catgagcagt 420
ctgcacctga aaagatatta tgggaggatt ctgcattacc tgaaggccaa ggagtacagt 480
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cttacaggtt acctccgaaa c 561

<210> 135
<211> 498
<212> DNA
<213> Homo sapiens

<400> 135
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ctgtggcaat tgaatgggag gcttgaatat tgcctcaagg acaggatgaa ctttgacatc 120
cctgaggaga ttaagcagct gcagcagttc cagaaggagg acgccgcatt gaccatctat 180
gagatgctcc agaacatctt tgctattttc agacaagatt catctagcac tggctggaat 240
gagactattg ttgagaacct cctggctaat gtctatcatc agataaacca tctgaagaca 300
gtcctggaag aaaaactgga gaaagaagat ttcaccaggg gaaaactcat gagcagtctg 360
cacctgaaaa gatattatgg gaggattctg cattacctga aggccaagga gtacagtcac 420
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acaggttacc tccgaaac 498

<210> 136
<211> 498
<212> DNA
<213> Homo sapiens

<400> 136
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ctgtggcaat tgaatgggag gcttgaatat tgcctcaagg acaggatgaa ctttgacatc 120
cctgaggaga ttaagcagct gcagcagttc cagaaggagg acgccgcatt gaccatctat 180
gagatgctcc agaacatctt tgctattttc agacaagatt catctagcac tggctggaat 240
gagactattg ttgagaacct cctggctaat gtctatcatc agataaacca tctgaagaca 300
gtcctggaag aaaaactgga gaaagaagat ttcaccaggg gaaaactcat gagcagtctg 360
cacctgaaaa gatattatgg gaggattctg cattacctga aggccaagga gtacagtcac 420
tgtgcctgga ccatagtcag agtggaaatc ctaaggaact tttacttcat taacagactt 480
acaggttacc tccgaaac 498

<210> 137
<211> 498
<212> DNA
<213> Homo sapiens

<400> 137
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cctgaggaga ttaagcagct gcagcagttc cagaaggagg acgccgcatt gaccatctat 180
gagatgctcc agaacatctt tgctattttc agacaagatt catctagcac tggctggaat 240
gagactattg ttgagaacct cctggctaat gtctatcatc agataaacca tctgaagaca 300
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cacctgaaaa gatattatgg gaggattctg cattacctga aggccaagga gtacagtcac 420
tgtgcctgga ccatagtcag agtggaaatc ctaaggaact tttacttcat taacagactt 480
acaggttacc tccgaaac 498

<210> 138
<211> 495
<212> DNA
<213> Homo sapiens

<400> 138
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gaggagtttg gcaaccagtt ccaaaaggct gaaaccatcc ctgtcctcca tgagatgatc 180
cagcagatct tcaatctctt cagcacaaag gactcatctg ctgcttggga tgagaccctc 240
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gaggttgtca gagcagaaat catgagatcc ctctctttat caacaaactt gcaagaaaga 480
ttaaggagga aggaa 495

<210> 139
<211> 495
<212> DNA
<213> Homo sapiens

<400> 139
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gaggagtttg gcaaccagtt ccaaaaggct gaaaccatcc ctgtcctcca tgagatgatc 180
cagcagatct tcaatctctt cagcacaaag gactcatctg ctgcttggga tgagaccctc 240
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aaatacttcc aaagaatcac tctatatctg acagagaaga aatacagctc ttgtgcctgg 420
gaggttgtca gagcagaaat catgagatcc ttctctttat caatcaactt gcaaaaaaga 480
ttgaagagta aggaa 495

<210> 140
<211> 495
<212> DNA
<213> Homo sapiens

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gaggagtttg gcaaccagtt ccaaaaggct gaaaccatcc ctgtcctcca tgagatgatc 180
cagcagatct tcaatctctt cagcacaaag gactcatctg ctgcttggga tgagaccctc 240
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ttaaggagga aggaa 495

<210> 141
<211> 495
<212> DNA
<213> Homo sapiens

<400> 141
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caggaggaga gggtgggaga aactcccctg atgaatgcgg actccatctt ggctgtgaag 360
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gaggttgtca gagcagaaat catgagatcc ctctctttat caacaaactt gcaagaaaga 480
ttaaggagga aggaa 495

<210> 142
<211> 495
<212> DNA
<213> Homo sapiens

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cagcagatct tcaatctctt cagcacaaag gactcatctg ctgcttggga tgagaccctc 240
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caggaggaga gggtgggaga aactcccctg atgaatgcgg actccatctt ggctgtgaag 360
aaatacttcc gaagaatcac tctctatctg acagagaaga aatacagccc ttgtgcctgg 420
gaggttgtca gagcagaaat catgagatcc ctctctttat caacaaactt gcaagaaaga 480
ttaaggagga aggaa 495

<210> 143
<211> 462
<212> DNA
<213> Homo sapiens

<400> 143
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ttacagatga ttttgaatgg aattaataat tacaagaatc ccaaactcac caggatgctc 180
acatttaagt tttacatgcc caagaaggcc acagaactga aacatcttca gtgtctagaa 240
gaagaactca aacctctgga ggaagtgcta aatttagctc aaagcaaaaa ctttcactta 300
agacccaggg acttaatcag caatatcaac gtaatagttc tggaactaaa gggatctgaa 360
acaacattca tgtgtgaata tgctgatgag acagcaacca ttgtagaatt tctgaacaga 420
tggattacct tttgtcaaag catcatctca acactgactt ga 462

<210> 144
<211> 462
<212> DNA
<213> Homo sapiens

<400> 144
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gcacctactt caagttctac aaagaaaaca cagctacaac tggagcattt actgctggat 120
ttacagatga ttttgaatgg aattaataat tacaagaatc ccaaactcac caggatgctc 180

246
247

acatttaagt tttacatgcc caagaaggcc acagaactga aacatcttca gtgtctagaa 240
gaagaactca aacctctgga ggaagtgcta aatttagctc aaagcaaaaa ctttcactta 300
agacccaggg acttaatcag caatatcaac gtaatagttc tggaactaaa gggatctgaa 360
acaacattca tgtgtgaata tgctgatgag acagcaacca ttgtagaatt tctgaacaga 420
tggattacct tttgtcaaag catcatctca acactgactt ga 462

<210> 145
<211> 462
<212> DNA
<213> Homo sapiens

<400> 145
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gcacctactt caagttctac aaagaaaaca cagctacaac tggagcattt actgctggat 120
ttacagatga ttttgaatgg aattaataat tacaagaatc ccaaactcac caggatgctc 180
acatttaagt tttacatgcc caagaaggcc acagaactga aacatcttca gtgtctagaa 240
gaagaactca aacctctgga ggaagtgcta aatttagctc aaagcaaaaa ctttcactta 300
agacccaggg acttaatcag caatatcaac gtaatagttc tggaactaaa gggatctgaa 360
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tggattacct tttgtcaaag catcatctca acactgactt ga 462

<210> 146
<211> 462
<212> DNA
<213> Homo sapiens

<400> 146
atgtacagga tgcaactcct gtcttgcatt gcactaagtc ttgcacttgt cacaaacagt 60
gcacctactt caagttctac aaagaaaaca cagctacaac tggagcattt actgctggat 120
ttacagatga ttttgaatgg aattaataat tacaagaatc ccaaactcac caggatgctc 180
acatttaagt tttacatgcc caagaaggcc acagaactga aacatcttca gtgtctagaa 240
gaagaactca aacctctgga ggaagtgcta aatttagctc aaagcaaaaa ctttcactta 300
agacccaggg acttaatcag caatatcaac gtaatagttc tggaactaaa gggatctgaa 360
acaacattca tgtgtgaata tgctgatgag acagcaacca ttgtagaatt tctgaacaga 420
tggattacct tttgtcaaag catcatctca acactgactt ga 462

<210> 147
<211> 453
<212> DNA
<213> Homo sapiens

<400> 147
atgcaactcc tgtcttgcat tgcactaagt cttgcacttg tcacaaacag tgcacctact 60
tcaagttcta caaagaaaac acagctacaa ctggagcatt tactgctgga tttacagatg 120
attttgaatg gaattaataa ttacaagaat cccaaactca ccaggatgct cacatttaag 180
ttttacatgc ccaagaaggc cacagaactg aaacatcttc agtgtctaga agaagaactc 240
aaacctctgg aggaagtgct aaatttagct caaagcaaaa actttcactt aagacccagg 300
gacttaatca gcaatatcaa cgtaatagtt ctggaactaa agggatctga aacaacattc 360
atgtgtgaat atgctgatga gacagcaacc attgtagaat ttctgaacag atggattacc 420
ttttctcaga gcatcatctc aacactgact tga 453

<210> 148
<211> 453
<212> DNA
<213> Homo sapiens

<400> 148

247
248

atgcaactcc tgtcttgcat tgcactaagt cttgcacttg tcacaaacag tgcacctact 60
tcaagttcta caaagaaaac acagctacaa ctggagcatt tactgctgga tttacagatg 120
attttgaatg gaattaataa ttacaagaat cccaaactca ccaggatgct cacatttaag 180
ttttacatgc ccaagaaggc cacagaactg aaacatcttc agtgtctaga agaagaactc 240
aaacctctgg aggaagtgct aaatttagct caaagcaaaa actttcactt aagacccagg 300
gacttaatca gcaatatcaa cgtaatagtt ctggaactaa agggatctga aacaacattc 360
atgtgtgaat atgctgatga gacagcaacc attgtagaat ttctgaacag atggattacc 420
ttttctcaga gcatcatctc aacactgact tga 453

<210> 149
<211> 767
<212> PRT
<213> Homo sapiens

<400> 149
Met Phe Lys Ser Val Val Tyr Ser Ile Leu Ala Ala Ser Leu Ala Asn
1 5 10 15

Ala Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly
20 25 30

Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu
35 40 45

Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr
50 55 60

Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp
65 70 75 80

Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr
85 90 95

Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu
100 105 110

Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn
115 120 125

Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe
130 135 140

His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala
145 150 155 160

Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys
165 170 175

Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala
180 185 190

Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala
195 200 205

Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly
210 215 220

Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe
225 230 235 240

Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr
245 250 255

Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp
260 265 270

Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile
275 280 285

Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser
290 295 300

His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro
305 310 315 320

Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr
325 330 335

Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala
340 345 350

Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys
355 360 365

Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His
370 375 380

Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu
385 390 395 400

Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly
405 410 415

Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val
420 425 430

Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly
435 440 445

Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro
450 455 460

Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu
465 470 475 480

His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu
485 490 495

Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu
500 505 510

Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala
515 520 525

Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr
530 535 540

Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln
545 550 555 560

Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys

565 570 575

Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu
580 585 590

Val Ala Ala Ser Gln Ala Ala Leu Gly Leu Cys Asp Leu Pro Gln Thr
595 600 605

His Ser Leu Gly Ser Arg Arg Thr Leu Met Leu Leu Ala Gln Met Arg
610 615 620

Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe
625 630 635 640

Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile Pro
645 650 655

Val Leu His Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys
660 665 670

Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Thr
675 680 685

Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Gly
690 695 700

Val Gly Val Thr Glu Thr Pro Leu Met Lys Glu Asp Ser Ile Leu Ala
705 710 715 720

Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Lys Glu Lys Lys
725 730 735

Tyr Ser Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser
740 745 750

Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu
755 760 765

<210> 150
<211> 769
<212> PRT
<213> Homo sapiens

<400> 150
Met Leu Leu Gln Ala Phe Leu Phe Leu Leu Ala Gly Phe Ala Ala Lys
1 5 10 15

Ile Ser Ala Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp
20 25 30

Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln
35 40 45

Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu
50 55 60

Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn
65 70 75 80

Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val

85 90 95

Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys
100 105 110

Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn
115 120 125

Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr
130 135 140

Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu
145 150 155 160

Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe
165 170 175

Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp
180 185 190

Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly,
195 200 205

Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys
210 215 220

Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln
225 230 235 240

Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp
245 250 255

Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys
260 265 270

Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp
275 280 285

Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu
290 295 300

Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp
305 310 315 320

Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys
325 330 335

Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu
340 345 350

Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu
355 360 365

Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp
370 375 380

Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val
385 390 395 400

Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln
405 410 415

Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys
420 425 430

Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn
435 440 445

Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg
450 455 460

Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys
465 470 475 480

Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys
485 490 495

Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val
500 505 510

Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe
515 520 525

His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys
530 535 540

Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys
545 550 555 560

Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys
565 570 575

Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys
580 585 590

Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu Cys Asp Leu Pro
595 600 605

Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met Leu Leu Ala Gln
610 615 620

Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp Arg His Asp Phe
625 630 635 640

Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr
645 650 655

Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser
660 665 670

Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe
675 680 685

Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile
690 695 700

Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys Glu Asp Ser Ile
705 710 715 720

Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Lys Glu
725 730 735

Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg*Ala Glu Ile Met
740 745 750

Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser Leu Arg Ser Lys
755 760 765

Glu

<210> 151
<211> 779
<212> PRT
<213> Homo sapiens

<400> 151
Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
1 5 10 15

Leu Glu His Thr His Arg Arg Gly Ser Leu Asp Lys Arg Asp Ala His
20 25 30

Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe
35 40 45

Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro
50 55 60

Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys
65 70 75 80

Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His
85 90 95

Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr
100 105 110

Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn
115 120 125

Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu
130 135 140

Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu
145 150 155 160

Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro
165 170 175

Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala
180 185 190

Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu
195 200 205

Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys
210 215 220

Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe
225 230 235 240

Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu
245 250 255

Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr
260 265 270

Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp
275 280 285

Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu
290 295 300

Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala
305 310 315 320

Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala
325 330 335

Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys
340 345 350

Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro
355 360 365

Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr
370 375 380

Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala
385 390 395 400

Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu
405 410 415

Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe
420 425 430

Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser
435 440 445

Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser
450 455 460

Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp
465 470 475 480

Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr
485 490 495

Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn
500 505 510

Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro
515 520 525

Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr
530 535 540

Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu
545 550 555 560

Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val

254
255

565 570 575

Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp
580 585 590

Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser
595 600 605

Gln Ala Ala Leu Gly Leu Cys Asp Leu Pro Gln Thr His Ser Leu Gly
610 615 620

Ser Arg Arg Thr Leu Met Leu Leu Ala Gln Met Arg Arg Ile Ser Leu
625 630 635 640

Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu
645 650 655

Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu
660 665 670

Met Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala
675 680 685

Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln
690 695 700

Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr
705 710 715 720

Glu Thr Pro Leu Met Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr
725 730 735

Phe Gln Arg Ile Thr Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys
740 745 750

Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser
755 760 765

Thr Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu
770 775

<210> 152
<211> 774
<212> PRT
<213> Homo sapiens

<400> 152
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Cys Asp Leu Pro Gln Thr His Ser
20 25 30

Leu Gly Ser Arg Arg Thr Leu Met Leu Leu Ala Gln Met Arg Arg Ile
35 40 45

Ser Leu Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln
50 55 60

Glu Glu Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile Pro Val Leu

65 70 75 80

His Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys Asp Ser
85 90 95

Ser Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Thr Glu Leu
100 105 110

Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Gly Val Gly
115 120 125

Val Thr Glu Thr Pro Leu Met Lys Glu Asp Ser Ile Leu Ala Val Arg
130 135 140

Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Lys Glu Lys Lys Tyr Ser
145 150 155 160

Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser
165 170 175

Leu Ser Thr Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu Asp Ala His
180 185 190

Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe
195 200 205

Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro
210 215 220

Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys
225 230 235 240

Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His
245 250 255

Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr
260 265 270

Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn
275 280 285

Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu
290 295 300

Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu
305 310 315 320

Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro
325 330 335

Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala
340 345 350

Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu
355 360 365

Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys
370 375 380

Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe
385 390 395 400

Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu
405 410 415

Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr
420 425 430

Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp
435 440 445

Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu
450 455 460

Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala
465 470 475 480

Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala
485 490 495

Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys
500 505 510

Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro
515 520 525

Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr
530 535 540

Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala
545 550 555 560

Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu
565 570 575

Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe
580 585 590

Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser
595 600 605

Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser
610 615 620

Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp
625 630 635 640

Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr
645 650 655

Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn
660 665 670

Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro
675 680 685

Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr
690 695 700

Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu
705 710 715 720

Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val
725 730 735

Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp
740 745 750

Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser
755 760 765

Gln Ala Ala Leu Gly Leu
770

<210> 153
<211> 769
<212> PRT
<213> Homo sapiens

<400> 153
Met Leu Leu Gln Ala Phe Leu Phe Leu Leu Ala Gly Phe Ala Ala Lys
1 5 10 15

Ile Ser Ala Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg
20 25 30

Thr Leu Met Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys
35 40 45

Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn
50 55 60

Gln Phe Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln
65 70 75 80

Gln Ile Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp
85 90 95

Glu Thr Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn
100 105 110

Asp Leu Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro
115 120 125

Leu Met Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg
130 135 140

Ile Thr Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu
145 150 155 160

Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu
165 170 175

Gln Glu Ser Leu Arg Ser Lys Glu Asp Ala His Lys Ser Glu Val Ala
180 185 190

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
195 200 205

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
210 215 220

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Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
225                 230                 235                 240

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
                245                 250                 255

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
            260                 265                 270

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
        275                 280                 285

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
    290                 295                 300

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
305                 310                 315                 320

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
                325                 330                 335

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
            340                 345                 350

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
        355                 360                 365

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
    370                 375                 380

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
385                 390                 395                 400

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
                405                 410                 415

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
            420                 425                 430

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
        435                 440                 445

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
    450                 455                 460

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
465                 470                 475                 480

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
                485                 490                 495

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
            500                 505                 510

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
        515                 520                 525

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
    530                 535                 540

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
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545 550 555 560

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
565 570 575

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
580 585 590

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
595 600 605

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
610 615 620

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
625 630 635 640

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
645 650 655

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
660 665 670

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
675 680 685

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
690 695 700

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
705 710 715 720

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
725 730 735

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
740 745 750

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
755 760 765

Leu

<210> 154
<211> 835
<212> PRT
<213> Homo sapiens

<400> 154
Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser
1 5 10 15

Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp Glu Thr Ala Gln
20 25 30

Ile Pro Ala Glu Ala Val Ile Gly Tyr Ser Asp Leu Glu Gly Asp Phe
35 40 45

Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu
50 55 60

```
Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val
 65                  70                  75                  80

Ser Leu Asp Lys Arg Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser
                 85                  90                  95

Arg Arg Thr Leu Met Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe
            100                 105                 110

Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe
        115                 120                 125

Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met
    130                 135                 140

Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala
145                 150                 155                 160

Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln
                165                 170                 175

Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu
            180                 185                 190

Thr Pro Leu Met Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe
        195                 200                 205

Gln Arg Ile Thr Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala
    210                 215                 220

Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr
225                 230                 235                 240

Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu Asp Ala His Lys Ser Glu
                245                 250                 255

Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu
            260                 265                 270

Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp
        275                 280                 285

His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val
    290                 295                 300

Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe
305                 310                 315                 320

Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu
                325                 330                 335

Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe
            340                 345                 350

Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro
        355                 360                 365

Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe
    370                 375                 380
```

Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr
385 390 395 400

Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr
405 410 415

Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu
420 425 430

Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu
435 440 445

Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp
450 455 460

Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu
465 470 475 480

Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys
485 490 495

His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys
500 505 510

Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys
515 520 525

Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu
530 535 540

Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val
545 550 555 560

Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe
565 570 575

Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser
580 585 590

Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu
595 600 605

Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe
610 615 620

Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln
625 630 635 640

Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala
645 650 655

Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr
660 665 670

Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys
675 680 685

Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser
690 695 700

Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser

705 710 715 720

Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro
725 730 735

Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe
740 745 750

Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu
755 760 765

Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys
770 775 780

His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp
785 790 795 800

Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr
805 810 815

Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala
820 825 830

Leu Gly Leu
835

<210> 155
<211> 774
<212> PRT
<213> Homo sapiens

<400> 155
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Gly Val Phe Arg Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys

263
264

145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu
610 615 620

Met Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys
625 630 635 640

Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe
645 650 655

Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile
660 665 670

Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
675 680 685

Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
690 695 700

Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met
705 710 715 720

Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
725 730 735

Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
740 745 750

Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
755 760 765

Ser Leu Arg Ser Lys Glu
770

<210> 156
<211> 773

<212> PRT
<213> Homo sapiens

<400> 156

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Met Ala Leu Thr Phe Ala Leu Leu Val Ala Leu Leu Val Leu Ser Cys
  1               5                  10                  15

Lys Ser Ser Cys Ser Val Gly Cys Asp Leu Pro Gln Thr His Ser Leu
             20                  25                  30

Gly Ser Arg Arg Thr Leu Met Leu Leu Ala Gln Met Arg Arg Ile Ser
         35                  40                  45

Leu Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu
     50                  55                  60

Glu Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile Pro Val Leu His
 65                  70                  75                  80

Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser
                 85                  90                  95

Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr
            100                 105                 110

Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Gly Val Gly Val
        115                 120                 125

Thr Glu Thr Pro Leu Met Lys Glu Asp Ser Ile Leu Ala Val Arg Lys
    130                 135                 140

Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro
145                 150                 155                 160

Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu
                165                 170                 175

Ser Thr Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu Asp Ala His Lys
            180                 185                 190

Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys
        195                 200                 205

Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe
    210                 215                 220

Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr
225                 230                 235                 240

Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr
                245                 250                 255

Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr
            260                 265                 270

Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu
        275                 280                 285

Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val
    290                 295                 300
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Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu
305                 310                 315                 320

Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr
                325                 330                 335

Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala
            340                 345                 350

Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro
        355                 360                 365

Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln
    370                 375                 380

Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys
385                 390                 395                 400

Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe
                405                 410                 415

Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu
            420                 425                 430

Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu
        435                 440                 445

Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys
    450                 455                 460

Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu
465                 470                 475                 480

Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp
                485                 490                 495

Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp
            500                 505                 510

Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp
        515                 520                 525

Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr
    530                 535                 540

Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys
545                 550                 555                 560

Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile
                565                 570                 575

Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln
            580                 585                 590

Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr
        595                 600                 605

Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys
    610                 615                 620

Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr
```

625 630 635 640

Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro
645 650 655

Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg
660 665 670

Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys
675 680 685

Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu
690 695 700

Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu
705 710 715 720

Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met
725 730 735

Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys
740 745 750

Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln
755 760 765

Ala Ala Leu Gly Leu
770

<210> 157
<211> 769
<212> PRT
<213> Homo sapiens

<400> 157
Met Leu Leu Gln Ala Phe Leu Phe Leu Leu Ala Gly Phe Ala Ala Lys
1 5 10 15

Ile Ser Ala Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg
20 25 30

Thr Leu Met Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys
35 40 45

Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn
50 55 60

Gln Phe Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln
65 70 75 80

Gln Ile Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp
85 90 95

Glu Thr Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn
100 105 110

Asp Leu Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro
115 120 125

Leu Met Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg

```
    130                 135                 140

Ile Thr Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu
145                 150                 155                 160

Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu
                165                 170                 175

Gln Glu Ser Leu Arg Ser Lys Glu Asp Ala His Lys Ser Glu Val Ala
            180                 185                 190

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
        195                 200                 205

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
    210                 215                 220

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
225                 230                 235                 240

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
                245                 250                 255

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
            260                 265                 270

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
        275                 280                 285

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
    290                 295                 300

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
305                 310                 315                 320

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
                325                 330                 335

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
            340                 345                 350

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
        355                 360                 365

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
    370                 375                 380

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
385                 390                 395                 400

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
                405                 410                 415

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
            420                 425                 430

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
        435                 440                 445

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
    450                 455                 460
```

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
465 470 475 480

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
485 490 495

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
500 505 510

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
515 520 525

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
530 535 540

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
545 550 555 560

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
565 570 575

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
580 585 590

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
595 600 605

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
610 615 620

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
625 630 635 640

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
645 650 655

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
660 665 670

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
675 680 685

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
690 695 700

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
705 710 715 720

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
725 730 735

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
740 745 750

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
755 760 765

Leu

<210> 158
<211> 774
<212> PRT
<213> Homo sapiens

<400> 158

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Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
  1               5                  10                  15

Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
             20                  25                  30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
         35                  40                  45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
     50                  55                  60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
 65                  70                  75                  80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
                 85                  90                  95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
            100                 105                 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
        115                 120                 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
    130                 135                 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145                 150                 155                 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
                165                 170                 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
            180                 185                 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
        195                 200                 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
    210                 215                 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225                 230                 235                 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
                245                 250                 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
            260                 265                 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
        275                 280                 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
```

271

```
    290                 295                 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305                 310                 315                 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
                325                 330                 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
            340                 345                 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
        355                 360                 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
    370                 375                 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385                 390                 395                 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
                405                 410                 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
            420                 425                 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
        435                 440                 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
    450                 455                 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465                 470                 475                 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
                485                 490                 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
            500                 505                 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
        515                 520                 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
    530                 535                 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545                 550                 555                 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
                565                 570                 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
            580                 585                 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
        595                 600                 605

Leu Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu
    610                 615                 620
```

Met Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys
625 630 635 640

Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe
645 650 655

Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile
660 665 670

Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
675 680 685

Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
690 695 700

Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met
705 710 715 720

Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
725 730 735

Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
740 745 750

Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
755 760 765

Ser Leu Arg Ser Lys Glu
770

<210> 159
<211> 775
<212> PRT
<213> Homo sapiens

<400> 159
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Met Ser Tyr Asn Leu Leu Gly Phe
20 25 30

Leu Gln Arg Ser Ser Asn Phe Gln Cys Gln Lys Leu Leu Trp Gln Leu
35 40 45

Asn Gly Arg Leu Glu Tyr Cys Leu Lys Asp Arg Met Asn Phe Asp Ile
50 55 60

Pro Glu Glu Ile Lys Gln Leu Gln Gln Phe Gln Lys Glu Asp Ala Ala
65 70 75 80

Leu Thr Ile Tyr Glu Met Leu Gln Asn Ile Phe Ala Ile Phe Arg Gln
85 90 95

Asp Ser Ser Ser Thr Gly Trp Asn Glu Thr Ile Val Glu Asn Leu Leu
100 105 110

Ala Asn Val Tyr His Gln Ile Asn His Leu Lys Thr Val Leu Glu Glu
115 120 125

```
Lys Leu Glu Lys Glu Asp Phe Thr Arg Gly Lys Leu Met Ser Ser Leu
    130                 135                 140

His Leu Lys Arg Tyr Tyr Gly Arg Ile Leu His Tyr Leu Lys Ala Lys
145                 150                 155                 160

Glu Tyr Ser His Cys Ala Trp Thr Ile Val Arg Val Glu Ile Leu Arg
                165                 170                 175

Asn Phe Tyr Phe Ile Asn Arg Leu Thr Gly Tyr Leu Arg Asn Asp Ala
            180                 185                 190

His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn
        195                 200                 205

Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys
    210                 215                 220

Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala
225                 230                 235                 240

Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu
                245                 250                 255

His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu
            260                 265                 270

Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg
        275                 280                 285

Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg
    290                 295                 300

Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn
305                 310                 315                 320

Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His
                325                 330                 335

Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys
            340                 345                 350

Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu
        355                 360                 365

Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala
    370                 375                 380

Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala
385                 390                 395                 400

Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala
                405                 410                 415

Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His
            420                 425                 430

Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala
        435                 440                 445
```

Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys
450 455 460

Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile
465 470 475 480

Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala
485 490 495

Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala
500 505 510

Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His
515 520 525

Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu
530 535 540

Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr
545 550 555 560

Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn
565 570 575

Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys
580 585 590

Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val
595 600 605

Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly
610 615 620

Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu
625 630 635 640

Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys
645 650 655

Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val
660 665 670

Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val
675 680 685

Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys
690 695 700

Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val
705 710 715 720

Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala
725 730 735

Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp
740 745 750

Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala
755 760 765

Ser Gln Ala Ala Leu Gly Leu

770 775

<210> 160
<211> 775
<212> PRT
<213> Homo sapiens

<400> 160
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile

```
        275                 280                 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
    290                 295                 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305                 310                 315                 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
                325                 330                 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
            340                 345                 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
        355                 360                 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
    370                 375                 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385                 390                 395                 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
                405                 410                 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
            420                 425                 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
        435                 440                 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
    450                 455                 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465                 470                 475                 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
                485                 490                 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
            500                 505                 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
        515                 520                 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
    530                 535                 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545                 550                 555                 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
                565                 570                 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
            580                 585                 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
        595                 600                 605
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Leu Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe
610 615 620

Gln Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys
625 630 635 640

Leu Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu
645 650 655

Gln Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu
660 665 670

Gln Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp
675 680 685

Asn Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile
690 695 700

Asn His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe
705 710 715 720

Thr Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly
725 730 735

Arg Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp
740 745 750

Thr Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg
755 760 765

Leu Thr Gly Tyr Leu Arg Asn
770 775

<210> 161
<211> 772
<212> PRT
<213> Homo sapiens

<400> 161
Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser
1 5 10 15

Thr Thr Ala Leu Ser Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg
20 25 30

Ser Ser Asn Phe Gln Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg
35 40 45

Leu Glu Tyr Cys Leu Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu
50 55 60

Ile Lys Gln Leu Gln Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile
65 70 75 80

Tyr Glu Met Leu Gln Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser
85 90 95

Ser Thr Gly Trp Asn Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val
100 105 110

Tyr His Gln Ile Asn His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu
115 120 125

Lys Glu Asp Phe Thr Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys
130 135 140

Arg Tyr Tyr Gly Arg Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser
145 150 155 160

His Cys Ala Trp Thr Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr
165 170 175

Phe Ile Asn Arg Leu Thr Gly Tyr Leu Arg Asn Asp Ala His Lys Ser
180 185 190

Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala
195 200 205

Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu
210 215 220

Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys
225 230 235 240

Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu
245 250 255

Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly
260 265 270

Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys
275 280 285

Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg
290 295 300

Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr
305 310 315 320

Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe
325 330 335

Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe
340 345 350

Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys
355 360 365

Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg
370 375 380

Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala
385 390 395 400

Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala
405 410 415

Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys
420 425 430

```
Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala
        435                 440                 445

Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu
    450                 455                 460

Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val
465                 470                 475                 480

Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe
                485                 490                 495

Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val
            500                 505                 510

Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr
        515                 520                 525

Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu
    530                 535                 540

Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val
545                 550                 555                 560

Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys
                565                 570                 575

Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn
            580                 585                 590

Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro
        595                 600                 605

Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys
    610                 615                 620

Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu
625                 630                 635                 640

Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val
                645                 650                 655

Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg
            660                 665                 670

Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu
        675                 680                 685

Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser
    690                 695                 700

Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val
705                 710                 715                 720

Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp
                725                 730                 735

Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu
            740                 745                 750

Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala
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755 760 765

Ala Leu Gly Leu
770

<210> 162
<211> 775
<212> PRT
<213> Homo sapiens

<400> 162
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Gly Val Phe Arg Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly

260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe
610 615 620

Gln Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys
625 630 635 640

Leu Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu
645 650 655

Gln Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu
660 665 670

Gln Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp
675 680 685

Asn Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile
690 695 700

Asn His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe
705 710 715 720

Thr Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly
725 730 735

Arg Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp
740 745 750

Thr Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg
755 760 765

Leu Thr Gly Tyr Leu Arg Asn
770 775

<210> 163
<211> 772
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<222> (240)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> MISC_FEATURE
<222> (270)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 163
Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser
1 5 10 15

Thr Thr Ala Leu Ser Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg
20 25 30

Ser Ser Asn Phe Gln Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg

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         35                  40                  45

Leu Glu Tyr Cys Leu Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu
     50                  55                  60

Ile Lys Gln Leu Gln Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile
 65                  70                  75                  80

Tyr Glu Met Leu Gln Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser
                 85                  90                  95

Ser Thr Gly Trp Asn Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val
            100                 105                 110

Tyr His Gln Ile Asn His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu
        115                 120                 125

Lys Glu Asp Phe Thr Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys
    130                 135                 140

Arg Tyr Tyr Gly Arg Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser
145                 150                 155                 160

His Cys Ala Trp Thr Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr
                165                 170                 175

Phe Ile Asn Arg Leu Thr Gly Tyr Leu Arg Asn Asp Ala His Lys Ser
            180                 185                 190

Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala
        195                 200                 205

Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu
    210                 215                 220

Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Xaa
225                 230                 235                 240

Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu
                245                 250                 255

Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Xaa Tyr Gly
            260                 265                 270

Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys
        275                 280                 285

Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg
    290                 295                 300

Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr
305                 310                 315                 320

Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe
                325                 330                 335

Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe
            340                 345                 350

Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys
        355                 360                 365
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Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg
370 375 380

Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala
385 390 395 400

Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala
405 410 415

Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys
420 425 430

Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala
435 440 445

Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu
450 455 460

Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val
465 470 475 480

Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe
485 490 495

Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val
500 505 510

Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr
515 520 525

Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu
530 535 540

Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val
545 550 555 560

Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys
565 570 575

Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn
580 585 590

Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro
595 600 605

Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys
610 615 620

Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu
625 630 635 640

Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val
645 650 655

Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg
660 665 670

Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu
675 680 685

Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser
690 695 700

Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val
705 710 715 720

Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp
725 730 735

Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu
740 745 750

Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala
755 760 765

Ala Leu Gly Leu
770

<210> 164
<211> 775
<212> PRT
<213> Homo sapiens

<220>
<221> MISC_FEATURE
<222> (487)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 164
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Gly Val Phe Arg Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro

165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Xaa His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe
610 615 620

Gln Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys
625 630 635 640

Leu Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu
645 650 655

Gln Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu
660 665 670

Gln Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp
675 680 685

Asn Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile
690 695 700

Asn His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe
705 710 715 720

Thr Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly
725 730 735

Arg Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp
740 745 750

Thr Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg
755 760 765

Leu Thr Gly Tyr Leu Arg Asn
770 775

<210> 165
<211> 771
<212> PRT
<213> Homo sapiens

<400> 165

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Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser
 1               5                  10                  15

Thr Thr Ala Leu Ser Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser
            20                  25                  30

Ser Asn Phe Gln Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu
        35                  40                  45

Glu Tyr Cys Leu Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile
    50                  55                  60

Lys Gln Leu Gln Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr
65                  70                  75                  80

Glu Met Leu Gln Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser
                85                  90                  95

Thr Gly Trp Asn Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr
            100                 105                 110

His Gln Ile Asn His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys
        115                 120                 125

Glu Asp Phe Thr Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg
    130                 135                 140

Tyr Tyr Gly Arg Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His
145                 150                 155                 160

Cys Ala Trp Thr Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe
                165                 170                 175

Ile Asn Arg Leu Thr Gly Tyr Leu Arg Asn Asp Ala His Lys Ser Glu
            180                 185                 190

Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu
        195                 200                 205

Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp
    210                 215                 220

His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val
225                 230                 235                 240

Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe
                245                 250                 255

Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu
            260                 265                 270

Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe
        275                 280                 285

Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro
    290                 295                 300

Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe
305                 310                 315                 320
```

Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr
325 330 335

Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr
340 345 350

Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu
355 360 365

Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu
370 375 380

Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp
385 390 395 400

Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu
405 410 415

Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys
420 425 430

His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys
435 440 445

Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys
450 455 460

Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu
465 470 475 480

Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val
485 490 495

Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe
500 505 510

Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser
515 520 525

Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu
530 535 540

Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe
545 550 555 560

Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln
565 570 575

Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala
580 585 590

Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr
595 600 605

Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys
610 615 620

Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser
625 630 635 640

Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser



645 650 655

Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro
660 665 670

Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe
675 680 685

Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu
690 695 700

Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys
705 710 715 720

His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp
725 730 735

Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr
740 745 750

Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala
755 760 765

Leu Gly Leu
770

<210> 166
<211> 771
<212> PRT
<213> Homo sapiens

<400> 166
Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser
1 5 10 15

Thr Thr Ala Leu Ser Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser
20 25 30

Ser Asn Phe Gln Ser Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu
35 40 45

Glu Tyr Cys Leu Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile
50 55 60

Lys Gln Leu Gln Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr
65 70 75 80

Glu Met Leu Gln Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser
85 90 95

Thr Gly Trp Asn Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr
100 105 110

His Gln Ile Asn His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys
115 120 125

Glu Asp Phe Thr Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg
130 135 140

Tyr Tyr Gly Arg Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His

145 150 155 160

Cys Ala Trp Thr Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe
165 170 175

Ile Asn Arg Leu Thr Gly Tyr Leu Arg Asn Asp Ala His Lys Ser Glu
180 185 190

Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu
195 200 205

Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp
210 215 220

His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val
225 230 235 240

Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe
245 250 255

Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu
260 265 270

Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe
275 280 285

Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro
290 295 300

Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe
305 310 315 320

Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr
325 330 335

Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr
340 345 350

Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu
355 360 365

Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu
370 375 380

Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp
385 390 395 400

Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu
405 410 415

Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys
420 425 430

His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys
435 440 445

Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys
450 455 460

Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu
465 470 475 480

```
Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val
                485                 490                 495

Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe
            500                 505                 510

Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser
        515                 520                 525

Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu
    530                 535                 540

Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe
545                 550                 555                 560

Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln
                565                 570                 575

Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala
            580                 585                 590

Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr
        595                 600                 605

Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys
    610                 615                 620

Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser
625                 630                 635                 640

Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser
                645                 650                 655

Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro
            660                 665                 670

Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe
        675                 680                 685

Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu
    690                 695                 700

Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys
705                 710                 715                 720

His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp
                725                 730                 735

Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr
            740                 745                 750

Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala
        755                 760                 765

Leu Gly Leu
    770
```

<210> 167
<211> 769

<212> PRT
<213> Homo sapiens

<400> 167

```
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ala Gly Val
  1               5                  10                  15

Ser Gly Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu
             20                  25                  30

Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr
         35                  40                  45

Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val
     50                  55                  60

Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys
 65                  70                  75                  80

Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala
                 85                  90                  95

Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln
            100                 105                 110

Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro
        115                 120                 125

Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala
    130                 135                 140

Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile
145                 150                 155                 160

Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala
                165                 170                 175

Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys
            180                 185                 190

Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys
        195                 200                 205

Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe
    210                 215                 220

Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg
225                 230                 235                 240

Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu
                245                 250                 255

Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala
            260                 265                 270

Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser
        275                 280                 285

Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys
    290                 295                 300
```

Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu
305 310 315 320

Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn
325 330 335

Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr
340 345 350

Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala
355 360 365

Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro
370 375 380

His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu
385 390 395 400

Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu
405 410 415

Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys
420 425 430

Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu
435 440 445

Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met
450 455 460

Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val
465 470 475 480

Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr
485 490 495

Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp
500 505 510

Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His
515 520 525

Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln
530 535 540

Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu
545 550 555 560

Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys
565 570 575

Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys
580 585 590

Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu Met Ser Tyr Asn Leu
595 600 605

Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln Cys Gln Lys Leu Leu
610 615 620

Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu Lys Asp Arg Met Asn

625 630 635 640

Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln Gln Phe Gln Lys Glu
645 650 655

Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln Asn Ile Phe Ala Ile
660 665 670

Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn Glu Thr Ile Val Glu
675 680 685

Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn His Leu Lys Thr Val
690 695 700

Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr Arg Gly Lys Leu Met
705 710 715 720

Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg Ile Leu His Tyr Leu
725 730 735

Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr Ile Val Arg Val Glu
740 745 750

Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu Thr Gly Tyr Leu Arg
755 760 765

Asn

<210> 168
<211> 769
<212> PRT
<213> Homo sapiens

<400> 168
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Gly Val
1 5 10 15

Ser Gly Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu
20 25 30

Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr
35 40 45

Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val
50 55 60

Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys
65 70 75 80

Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala
85 90 95

Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln
100 105 110

Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro
115 120 125

Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala
130 135 140

```
Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile
145                 150                 155                 160

Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala
                165                 170                 175

Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys
            180                 185                 190

Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys
        195                 200                 205

Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe
    210                 215                 220

Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg
225                 230                 235                 240

Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu
                245                 250                 255

Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala
            260                 265                 270

Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser
        275                 280                 285

Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys
    290                 295                 300

Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu
305                 310                 315                 320

Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn
                325                 330                 335

Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr
            340                 345                 350

Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala
        355                 360                 365

Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro
    370                 375                 380

His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu
385                 390                 395                 400

Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu
                405                 410                 415

Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys
            420                 425                 430

Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu
        435                 440                 445

Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met
    450                 455                 460
```

Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val
465 470 475 480

Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr
485 490 495

Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp
500 505 510

Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His
515 520 525

Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln
530 535 540

Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu
545 550 555 560

Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys
565 570 575

Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys
580 585 590

Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu Met Ser Tyr Asn Leu
595 600 605

Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln Cys Gln Lys Leu Leu
610 615 620

Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu Lys Asp Arg Met Asn
625 630 635 640

Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln Gln Phe Gln Lys Glu
645 650 655

Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln Asn Ile Phe Ala Ile
660 665 670

Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn Glu Thr Ile Val Glu
675 680 685

Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn His Leu Lys Thr Val
690 695 700

Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr Arg Gly Lys Leu Met
705 710 715 720

Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg Ile Leu His Tyr Leu
725 730 735

Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr Ile Val Arg Val Glu
740 745 750

Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu Thr Gly Tyr Leu Arg
755 760 765

Asn

<210> 169

297
299

<211> 769
<212> PRT
<213> Homo sapiens

<400> 169

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Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Gly Gly Val
  1               5                  10                  15

Ser Gly Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu
             20                  25                  30

Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr
         35                  40                  45

Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val
     50                  55                  60

Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys
 65                  70                  75                  80

Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala
                 85                  90                  95

Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln
            100                 105                 110

Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro
        115                 120                 125

Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala
    130                 135                 140

Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile
145                 150                 155                 160

Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala
                165                 170                 175

Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys
            180                 185                 190

Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys
        195                 200                 205

Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe
    210                 215                 220

Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg
225                 230                 235                 240

Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu
                245                 250                 255

Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala
            260                 265                 270

Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser
        275                 280                 285

Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys
    290                 295                 300
```

Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu
305 310 315 320

Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn
325 330 335

Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr
340 345 350

Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala
355 360 365

Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro
370 375 380

His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu
385 390 395 400

Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu
405 410 415

Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys
420 425 430

Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu
435 440 445

Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met
450 455 460

Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val
465 470 475 480

Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr
485 490 495

Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp
500 505 510

Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His
515 520 525

Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln
530 535 540

Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu
545 550 555 560

Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys
565 570 575

Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys
580 585 590

Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu Met Ser Tyr Asn Leu
595 600 605

Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln Cys Gln Lys Leu Leu
610 615 620

299
301

```
Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu Lys Asp Arg Met Asn
625                 630                 635                 640

Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln Gln Phe Gln Lys Glu
                645                 650                 655

Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln Asn Ile Phe Ala Ile
            660                 665                 670

Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn Glu Thr Ile Val Glu
        675                 680                 685

Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn His Leu Lys Thr Val
    690                 695                 700

Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr Arg Gly Lys Leu Met
705                 710                 715                 720

Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg Ile Leu His Tyr Leu
                725                 730                 735

Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr Ile Val Arg Val Glu
            740                 745                 750

Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu Thr Gly Tyr Leu Arg
        755                 760                 765

Asn
```

<210> 170
<211> 774
<212> PRT
<213> Homo sapiens

<400> 170

```
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
  1               5                  10                  15

Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
             20                  25                  30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
         35                  40                  45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
     50                  55                  60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
 65                  70                  75                  80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
                 85                  90                  95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
            100                 105                 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
        115                 120                 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
```

130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

```
Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465                 470                 475                 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
                485                 490                 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
            500                 505                 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
        515                 520                 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
    530                 535                 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545                 550                 555                 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
                565                 570                 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
            580                 585                 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
        595                 600                 605

Leu Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu
    610                 615                 620

Met Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys
625                 630                 635                 640

Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe
                645                 650                 655

Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile
            660                 665                 670

Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
        675                 680                 685

Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
    690                 695                 700

Glu Ala Cys Val Met Gln Glu Glu Arg Val Gly Glu Thr Pro Leu Met
705                 710                 715                 720

Asn Ala Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Arg Arg Ile Thr
                725                 730                 735

Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
            740                 745                 750

Arg Ala Glu Ile Met Arg Ser Leu Ser Leu Ser Thr Asn Leu Gln Glu
        755                 760                 765

Arg Leu Arg Arg Lys Glu
    770
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<210> 171
<211> 774
<212> PRT
<213> Homo sapiens

<400> 171

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Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
  1               5                  10                  15

Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
             20                  25                  30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
         35                  40                  45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
     50                  55                  60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
 65                  70                  75                  80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
                 85                  90                  95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
            100                 105                 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
        115                 120                 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
    130                 135                 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145                 150                 155                 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
                165                 170                 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
            180                 185                 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
        195                 200                 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
    210                 215                 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225                 230                 235                 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
                245                 250                 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
            260                 265                 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
        275                 280                 285
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Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu

610 615 620

Met Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys
625 630 635 640

Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe
645 650 655

Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile
660 665 670

Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
675 680 685

Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
690 695 700

Glu Ser Cys Val Met Gln Glu Val Gly Val Ile Glu Ser Pro Leu Met
705 710 715 720

Tyr Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
725 730 735

Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Ser Cys Ala Trp Glu Val Val
740 745 750

Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Ile Asn Leu Gln Lys
755 760 765

Arg Leu Lys Ser Lys Glu
770

<210> 172
<211> 774
<212> PRT
<213> Homo sapiens

<400> 172
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln

115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu
610 615 620

Met Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys
625 630 635 640

Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe
645 650 655

Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile
660 665 670

Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
675 680 685

Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Met
690 695 700

Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu Met
705 710 715 720

Asn Val Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Gln Arg Ile Thr
725 730 735

Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
740 745 750

Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Lys Ile Phe Gln Glu
755 760 765

Arg Leu Arg Arg Lys Glu
770

<210> 173
<211> 774
<212> PRT
<213> Homo sapiens

<400> 173
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

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Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
        275                 280                 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
    290                 295                 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305                 310                 315                 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
                325                 330                 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
            340                 345                 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
        355                 360                 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
    370                 375                 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385                 390                 395                 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
                405                 410                 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
            420                 425                 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
        435                 440                 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
    450                 455                 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465                 470                 475                 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
                485                 490                 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
            500                 505                 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
        515                 520                 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
    530                 535                 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545                 550                 555                 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
                565                 570                 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
            580                 585                 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
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595 600 605

Leu Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu
610 615 620

Met Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys
625 630 635 640

Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe
645 650 655

Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile
660 665 670

Phe Asn Leu Phe Thr Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Asp
675 680 685

Leu Leu Asp Lys Phe Cys Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
690 695 700

Glu Ala Cys Val Met Gln Glu Glu Arg Val Gly Glu Thr Pro Leu Met
705 710 715 720

Asn Ala Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Arg Arg Ile Thr
725 730 735

Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
740 745 750

Arg Ala Glu Ile Met Arg Ser Leu Ser Leu Ser Thr Asn Leu Gln Glu
755 760 765

Arg Leu Arg Arg Lys Glu
770

<210> 174
<211> 774
<212> PRT
<213> Homo sapiens

<400> 174
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala

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            100                 105                 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
        115                 120                 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
    130                 135                 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145                 150                 155                 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
                165                 170                 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
            180                 185                 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
        195                 200                 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
    210                 215                 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225                 230                 235                 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
                245                 250                 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
            260                 265                 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
        275                 280                 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
    290                 295                 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305                 310                 315                 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
                325                 330                 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
            340                 345                 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
        355                 360                 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
    370                 375                 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385                 390                 395                 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
                405                 410                 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
            420                 425                 430
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Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu
610 615 620

Met Leu Leu Ala Gln Met Arg Lys Ile Ser Leu Phe Ser Cys Leu Lys
625 630 635 640

Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe
645 650 655

Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile
660 665 670

Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
675 680 685

Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
690 695 700

Glu Ala Cys Val Met Gln Glu Glu Arg Val Gly Glu Thr Pro Leu Met
705 710 715 720

Asn Val Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Arg Arg Ile Thr
725 730 735

Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
740 745 750

Arg Ala Glu Ile Met Arg Ser Leu Ser Leu Ser Thr Asn Leu Gln Glu
755 760 765

Arg Leu Arg Arg Lys Glu
770

<210> 175
<211> 742
<212> PRT
<213> Homo sapiens

<400> 175
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Ala Pro Thr Ser Ser Ser Thr Lys
20 25 30

Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile
35 40 45

Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu
50 55 60

Thr Phe Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu
65 70 75 80

Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu
85 90 95

Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn
100 105 110

Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met
115 120 125

Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg
130 135 140

Trp Ile Thr Phe Ser Gln Ser Ile Ile Ser Thr Leu Thr Asp Ala His
145 150 155 160

Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe
165 170 175

Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro
180 185 190

Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys
195 200 205

Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His
210 215 220

Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr
225 230 235 240

Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn
245 250 255

Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu
260 265 270

Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu
275 280 285

Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro
290 295 300

Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala
305 310 315 320

Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu
325 330 335

Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys
340 345 350

Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe
355 360 365

Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu
370 375 380

Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr
385 390 395 400

Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp
405 410 415

Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu
420 425 430

Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala
435 440 445

Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala
450 455 460

Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys
465 470 475 480

Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro
485 490 495

Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr
500 505 510

Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala
515 520 525

Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu
530 535 540

Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe
545 550 555 560

Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser
565 570 575

Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser

580 585 590

Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp
595 600 605

Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr
610 615 620

Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn
625 630 635 640

Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro
645 650 655

Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr
660 665 670

Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu
675 680 685

Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val
690 695 700

Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp
705 710 715 720

Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser
725 730 735

Gln Ala Ala Leu Gly Leu
740

<210> 176
<211> 742
<212> PRT
<213> Homo sapiens

<400> 176
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln

115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu
610 615 620

His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr
625 630 635 640

Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro
645 650 655

Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu
660 665 670

Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His
675 680 685

Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu
690 695 700

Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr
705 710 715 720

Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Ser Gln Ser
725 730 735

Ile Ile Ser Thr Leu Thr
740

<210> 177
<211> 742

<212> PRT
<213> Homo sapiens

<400> 177

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Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
  1               5                  10                  15

Tyr Ser Arg Ser Leu Asp Lys Arg Ala Pro Thr Ser Ser Ser Thr Lys
             20                  25                  30

Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile
         35                  40                  45

Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu
     50                  55                  60

Thr Phe Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu
 65                  70                  75                  80

Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu
                 85                  90                  95

Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn
            100                 105                 110

Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met
        115                 120                 125

Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg
    130                 135                 140

Trp Ile Thr Phe Cys Gln Ser Ile Ile Ser Thr Leu Thr Asp Ala His
145                 150                 155                 160

Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe
                165                 170                 175

Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro
            180                 185                 190

Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys
        195                 200                 205

Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His
    210                 215                 220

Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr
225                 230                 235                 240

Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn
                245                 250                 255

Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu
            260                 265                 270

Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu
        275                 280                 285

Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro
    290                 295                 300
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Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala
305 310 315 320

Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu
325 330 335

Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys
340 345 350

Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe
355 360 365

Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu
370 375 380

Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr
385 390 395 400

Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp
405 410 415

Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu
420 425 430

Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala
435 440 445

Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala
450 455 460

Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys
465 470 475 480

Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro
485 490 495

Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr
500 505 510

Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala
515 520 525

Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu
530 535 540

Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe
545 550 555 560

Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser
565 570 575

Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser
580 585 590

Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp
595 600 605

Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr
610 615 620

Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn

625 630 635 640

Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro
645 650 655

Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr
660 665 670

Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu
675 680 685

Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val
690 695 700

Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp
705 710 715 720

Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser
725 730 735

Gln Ala Ala Leu Gly Leu
740

<210> 178
<211> 742
<212> PRT
<213> Homo sapiens

<400> 178
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro

165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu
610 615 620

His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr
625 630 635 640

Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro
645 650 655

Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu
660 665 670

Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His
675 680 685

Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu
690 695 700

Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr
705 710 715 720

Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser
725 730 735

Ile Ile Ser Thr Leu Thr
740

<210> 179
<211> 738
<212> PRT
<213> Homo sapiens

<400> 179
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
20 25 30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
35 40 45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe
50 55 60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
65 70 75 80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
85 90 95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
100 105 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
115 120 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
130 135 140

Cys Gln Ser Ile Ile Ser Thr Leu Thr Asp Ala His Lys Ser Glu Val
145 150 155 160

Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val
165 170 175

Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His
180 185 190

Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala
195 200 205

Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly
210 215 220

Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met
225 230 235 240

Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu
245 250 255

Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu
260 265 270

Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu
275 280 285

Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala
290 295 300

Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu
305 310 315 320

Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp
325 330 335

Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys
340 345 350

Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala
355 360 365

Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val
370 375 380

Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His
385 390 395 400

Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr
405 410 415

Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys
420 425 430

Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn
435 440 445

Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu
450 455 460

Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu
465 470 475 480

Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val
485 490 495

Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys
500 505 510

Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp
515 520 525

Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn
530 535 540

Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu
545 550 555 560

Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu
565 570 575

Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys
580 585 590

His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val
595 600 605

Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp
610 615 620

Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys
625 630 635 640

Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn
645 650 655

Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys
660 665 670

Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His

675 680 685

Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe
690 695 700

Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys
705 710 715 720

Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu
725 730 735

Gly Leu

<210> 180
<211> 738
<212> PRT
<213> Homo sapiens

<400> 180
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
20 25 30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
35 40 45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe
50 55 60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
65 70 75 80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
85 90 95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
100 105 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
115 120 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
130 135 140

Cys Gln Ser Ile Ile Ser Thr Leu Thr Asp Ala His Lys Ser Glu Val
145 150 155 160

Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val
165 170 175

Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His
180 185 190

Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala
195 200 205

Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly
210 215 220

Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met
225 230 235 240

Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu
245 250 255

Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu
260 265 270

Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu
275 280 285

Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala
290 295 300

Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu
305 310 315 320

Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp
325 330 335

Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys
340 345 350

Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala
355 360 365

Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val
370 375 380

Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His
385 390 395 400

Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr
405 410 415

Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys
420 425 430

Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn
435 440 445

Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu
450 455 460

Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu
465 470 475 480

Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val
485 490 495

Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys
500 505 510

Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp
515 520 525

Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn
530 535 540

Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu
545 550 555 560

Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu
565 570 575

Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys
580 585 590

His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val
595 600 605

Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp
610 615 620

Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys
625 630 635 640

Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn
645 650 655

Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys
660 665 670

Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His
675 680 685

Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe
690 695 700

Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys
705 710 715 720

Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu
725 730 735

Gly Leu

<210> 181
<211> 165
<212> PRT
<213> Homo sapiens

<400> 181
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu

85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> 182
<211> 165
<212> PRT
<213> Homo sapiens

<400> 182
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> 183
<211> 165
<212> PRT
<213> Homo sapiens

<400> 183
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> 184
<211> 165
<212> PRT
<213> Homo sapiens

<400> 184
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys

100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> 185
<211> 165
<212> PRT
<213> Homo sapiens

<400> 185
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> 186
<211> 165
<212> PRT
<213> Homo sapiens

<400> 186
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met

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 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> 187
<211> 165
<212> PRT
<213> Homo sapiens

<400> 187
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
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115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> 188
<211> 165
<212> PRT
<213> Homo sapiens

<400> 188
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> 189
<211> 165
<212> PRT
<213> Homo sapiens

<400> 189
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> 190
<211> 165
<212> PRT
<213> Homo sapiens

<400> 190
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> 191
<211> 166
<212> PRT
<213> Homo sapiens

<400> 191
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
1 5 10 15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
20 25 30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
35 40 45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
50 55 60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65 70 75 80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
85 90 95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
100 105 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
115 120 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
130 135 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145 150 155 160

Thr Gly Tyr Leu Arg Asn
165

<210> 192
<211> 166
<212> PRT
<213> Homo sapiens

<400> 192
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
1 5 10 15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
20 25 30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
35 40 45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
50 55 60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65 70 75 80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
85 90 95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
100 105 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
115 120 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
130 135 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145 150 155 160

Thr Gly Tyr Leu Arg Asn
165

<210> 193
<211> 187
<212> PRT
<213> Homo sapiens

<400> 193
Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser
1 5 10 15

Thr Thr Ala Leu Ser Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg
20 25 30

Ser Ser Asn Phe Gln Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg
35 40 45

Leu Glu Tyr Cys Leu Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu
50 55 60

Ile Lys Gln Leu Gln Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile
65 70 75 80

Tyr Glu Met Leu Gln Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser
85 90 95

Ser Thr Gly Trp Asn Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val
100 105 110

Tyr His Gln Ile Asn His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu
115 120 125

Lys Glu Asp Phe Thr Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys
130 135 140

Arg Tyr Tyr Gly Arg Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser
145 150 155 160

His Cys Ala Trp Thr Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr
165 170 175

Phe Ile Asn Arg Leu Thr Gly Tyr Leu Arg Asn
180 185

<210> 194
<211> 166
<212> PRT
<213> Homo sapiens

<400> 194
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
1 5 10 15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
20 25 30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
35 40 45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
50 55 60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65 70 75 80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
85 90 95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
100 105 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
115 120 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
130 135 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145 150 155 160

Thr Gly Tyr Leu Arg Asn
165

<210> 195
<211> 187
<212> PRT
<213> Homo sapiens

<400> 195
Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser
1 5 10 15

Thr Thr Ala Leu Ser Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg

20 25 30

Ser Ser Asn Phe Gln Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg
35 40 45

Leu Glu Tyr Cys Leu Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu
50 55 60

Ile Lys Gln Leu Gln Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile
65 70 75 80

Tyr Glu Met Leu Gln Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser
85 90 95

Ser Thr Gly Trp Asn Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val
100 105 110

Tyr His Gln Ile Asn His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu
115 120 125

Lys Glu Asp Phe Thr Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys
130 135 140

Arg Tyr Tyr Gly Arg Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser
145 150 155 160

His Cys Ala Trp Thr Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr
165 170 175

Phe Ile Asn Arg Leu Thr Gly Tyr Leu Arg Asn
180 185

<210> 196
<211> 166
<212> PRT
<213> Homo sapiens

<400> 196
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
1 5 10 15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
20 25 30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
35 40 45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
50 55 60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65 70 75 80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
85 90 95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
100 105 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg

115 120 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
130 135 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145 150 155 160

Thr Gly Tyr Leu Arg Asn
165

<210> 197
<211> 187
<212> PRT
<213> Homo sapiens

<400> 197
Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser
1 5 10 15

Thr Thr Ala Leu Ser Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg
20 25 30

Ser Ser Asn Phe Gln Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg
35 40 45

Leu Glu Tyr Cys Leu Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu
50 55 60

Ile Lys Gln Leu Gln Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile
65 70 75 80

Tyr Glu Met Leu Gln Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser
85 90 95

Ser Thr Gly Trp Asn Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val
100 105 110

Tyr His Gln Ile Asn His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu
115 120 125

Lys Glu Asp Phe Thr Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys
130 135 140

Arg Tyr Tyr Gly Arg Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser
145 150 155 160

His Cys Ala Trp Thr Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr
165 170 175

Phe Ile Asn Arg Leu Thr Gly Tyr Leu Arg Asn
180 185

<210> 198
<211> 187
<212> PRT
<213> Homo sapiens

<400> 198

Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser
1 5 10 15

Thr Thr Ala Leu Ser Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg
20 25 30

Ser Ser Asn Phe Gln Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg
35 40 45

Leu Glu Tyr Cys Leu Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu
50 55 60

Ile Lys Gln Leu Gln Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile
65 70 75 80

Tyr Glu Met Leu Gln Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser
85 90 95

Ser Thr Gly Trp Asn Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val
100 105 110

Tyr His Gln Ile Asn His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu
115 120 125

Lys Glu Asp Phe Thr Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys
130 135 140

Arg Tyr Tyr Gly Arg Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser
145 150 155 160

His Cys Ala Trp Thr Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr
165 170 175

Phe Ile Asn Arg Leu Thr Gly Tyr Leu Arg Asn
180 185

<210> 199
<211> 166
<212> PRT
<213> Homo sapiens

<400> 199
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
1 5 10 15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
20 25 30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
35 40 45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
50 55 60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65 70 75 80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
85 90 95

341 08

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His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165
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<210> 200
<211> 166
<212> PRT
<213> Homo sapiens

<400> 200

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Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
  1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
             20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
         35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
     50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
 65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                 85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165
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<210> 201
<211> 166
<212> PRT
<213> Homo sapiens

<400> 201
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
1 5 10 15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
20 25 30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
35 40 45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
50 55 60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65 70 75 80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
85 90 95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
100 105 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
115 120 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
130 135 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145 150 155 160

Thr Gly Tyr Leu Arg Asn
165

<210> 202
<211> 165
<212> PRT
<213> Homo sapiens

<400> 202
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Met Gln Glu Glu Arg Val Gly Glu Thr Pro Leu Met Asn
100 105 110

Ala Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Arg Arg Ile Thr Leu
115 120 125

Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Leu Ser Leu Ser Thr Asn Leu Gln Glu Arg
145 150 155 160

Leu Arg Arg Lys Glu
165

<210> 203
<211> 165
<212> PRT
<213> Homo sapiens

<400> 203
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ser Cys Val Met Gln Glu Val Gly Val Ile Glu Ser Pro Leu Met Tyr
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Thr Glu Lys Lys Tyr Ser Ser Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Ile Asn Leu Gln Lys Arg
145 150 155 160

Leu Lys Ser Lys Glu
165

<210> 204
<211> 165
<212> PRT
<213> Homo sapiens

<400> 204
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met

1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Met Glu
85 90 95

Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu Met Asn
100 105 110

Val Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Lys Ile Phe Gln Glu Arg
145 150 155 160

Leu Arg Arg Lys Glu
165

<210> 205
<211> 165
<212> PRT
<213> Homo sapiens

<400> 205
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Thr Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Asp Leu
65 70 75 80

Leu Asp Lys Phe Cys Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Met Gln Glu Glu Arg Val Gly Glu Thr Pro Leu Met Asn
100 105 110

Ala Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Arg Arg Ile Thr Leu

115 120 125

Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Leu Ser Leu Ser Thr Asn Leu Gln Glu Arg
145 150 155 160

Leu Arg Arg Lys Glu
165

<210> 206
<211> 165
<212> PRT
<213> Homo sapiens

<400> 206
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Met Gln Glu Glu Arg Val Gly Glu Thr Pro Leu Met Asn
100 105 110

Ala Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Arg Arg Ile Thr Leu
115 120 125

Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Leu Ser Leu Ser Thr Asn Leu Gln Glu Arg
145 150 155 160

Leu Arg Arg Lys Glu
165

<210> 207
<211> 153
<212> PRT
<213> Homo sapiens

<400> 207
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
20 25 30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
35 40 45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe
50 55 60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
65 70 75 80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
85 90 95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
100 105 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
115 120 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
130 135 140

Cys Gln Ser Ile Ile Ser Thr Leu Thr
145 150

<210> 208
<211> 153
<212> PRT
<213> Homo sapiens

<400> 208
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
20 25 30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
35 40 45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe
50 55 60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
65 70 75 80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
85 90 95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
100 105 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
115 120 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
130 135 140

346
347

Cys Gln Ser Ile Ile Ser Thr Leu Thr
145 150

<210> 209
<211> 153
<212> PRT
<213> Homo sapiens

<400> 209
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
20 25 30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
35 40 45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe
50 55 60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
65 70 75 80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
85 90 95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
100 105 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
115 120 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
130 135 140

Cys Gln Ser Ile Ile Ser Thr Leu Thr
145 150

<210> 210
<211> 153
<212> PRT
<213> Homo sapiens

<400> 210
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
20 25 30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
35 40 45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe
50 55 60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
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Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
85 90 95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
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Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
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Cys Gln Ser Ile Ile Ser Thr Leu Thr
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Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe
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Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
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Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
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Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
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Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
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35 40 45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe
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Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
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Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
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Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
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<213> Homo sapiens

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gaattcattg cttggttggt taaggggtaga 90

<210> 252
<211> 96
<212> DNA
<213> Homo sapiens

<400> 252
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tccagtggcc tgggctgcaa agtgctgagg cggcat 96

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<212> DNA
<213> Homo sapiens

<400> 253
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tccagtggcc tgggctgcaa agtgctgagg cggcat 96

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<211> 2004
<212> DNA
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<211> 87
<212> DNA
<213> Homo sapiens

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tccagtggcc tgggctgcaa agtgctg 87

<210> 256
<211> 96
<212> DNA
<213> Homo sapiens

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tccagtggcc tgggctgcaa agtgctgagg cggcat 96

<210> 257
<211> 96
<212> DNA
<213> Homo sapiens

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tccagtggcc tgggctgcaa agtgctgagg cggcat 96

<210> 258
<211> 96
<212> DNA
<213> Homo sapiens

<400> 258
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tccagtggcc tgggctgcaa agtgctgagg cggcat 96

<210> 259
<211> 96
<212> DNA
<213> Homo sapiens

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tccagtggcc tgggctgcaa agtgctgagg cggcat 96

<210> 260
<211> 96

<212> DNA
<213> Homo sapiens

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<211> 1716
<212> DNA
<213> Homo sapiens

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<211> 96
<212> DNA
<213> Homo sapiens

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<210> 263
<211> 96
<212> DNA
<213> Homo sapiens

<400> 263
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361/15

<210> 264
<211> 96
<212> DNA
<213> Homo sapiens

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<211> 1929
<212> DNA
<213> Homo sapiens

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<211> 456
<212> DNA
<213> Homo sapiens

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<211> 456
<212> DNA
<213> Homo sapiens

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cagagcggac tgggctgtaa cagcttccgg tactga 456

<210> 271
<211> 456
<212> DNA
<213> Homo sapiens

<400> 271
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cagagcggac tgggctgtaa cagcttccgg tactga 456

<210> 272
<211> 381
<212> DNA
<213> Homo sapiens

<400> 272
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atgagcggcc tgggatgtta g 381

<210> 273
<211> 381
<212> DNA
<213> Homo sapiens

<400> 273
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tcgcgggcag cgtgggctcg ccttctgcaa gagcacccca acgcgcgcaa atacaaagga 300
gccaacaaga agggcttgtc caagggctgc ttcggcctca agctggaccg aatcggctcc 360
atgagcggcc tgggatgtta g 381

<210> 274
<211> 114
<212> DNA
<213> Dendroaspis angusticeps

<400> 274
gaagttaagt acgatccatg tttcggtcac aagattgata gaattaacca cgtttctaac 60
ttgggttgtc catctttgag agatccaaga ccaaacgctc catctacttc tgct 114

<210> 275
<211> 114
<212> DNA
<213> Dendroaspis angusticeps

<400> 275
gaagttaagt acgatccatg tttcggtcac aagattgata gaattaacca cgtttctaac 60

ttgggttgtc catctttgag agatccaaga ccaaacgctc catctacttc tgct 114

<210> 276
<211> 1929
<212> DNA
<213> Homo sapiens

<400> 276
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ggcttataa 1929

<210> 277
<211> 96
<212> DNA
<213> Homo sapiens

<400> 277
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tccagtggcc tgggctgcaa agtgctgagg cggcat 96

<210> 278
<211> 96
<212> DNA
<213> Homo sapiens

<400> 278
agccccaaga tggtgcaagg gtctggctgc tttgggaggg gcatggaccg gatcagctcc 60
tccagtggcc tgggctgcaa agtgctgagg cggcat 96

<210> 279

<211> 96
<212> DNA
<213> Homo sapiens

<400> 279
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tccagtggcc tgggctgcaa agtgctgagg cggcat 96

<210> 280
<211> 96
<212> DNA
<213> Homo sapiens

<400> 280
agccccaaga tggtgcaagg gtctggctgc tttgggaggg gcatggaccg gatcagctcc 60
tccagtggcc tgggctgcaa agtgctgagg cggcat 96

<210> 281
<211> 912
<212> DNA
<213> Rattus norvegicus

<400> 281
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tatgggaagg acatggtcaa agttctccat attcagaggg atggaaaata ccacagcatc 120
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ttgctgccctc tggacaaccc ctacggcaaa ataaccggga cggtgaggag gaagctgcct 900
tccaggctgt ga 912

<210> 282
<211> 912
<212> DNA
<213> Rattus norvegicus

<400> 282
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tatgggaagg acatggtcaa agttctccat attcagaggg atggaaaata ccacagcatc 120
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tccaggctgt ga 912

<210> 283
<211> 1233
<212> DNA
<213> Mycoplasma arginini

<400> 283
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gatgaattat tattctcagc tatcttagaa agccacgatg ctagaaaaga acacaaacaa 180
ttcgtagcag aattaaaagc aaacgacatc aatgttgttg aattaattga tttagttgct 240
gaaacatatg atttagcatc acaagaagct aaagataaat taatcgaaga atttttagaa 300
gactcagaac cagttctatc agaagaacac aaagtagttg taagaaactt cttaaaagct 360
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ggtatcgaag cagatcacga attaatcgtt gacccaatgc caaacctata cttcacacgt 480
gacccatttg catcagtagg taatggtgta acaatccact acatgcgtta caaagttaga 540
caacgtgaaa cattattctc aagatttgta ttctcaaatc accctaaact aattaacact 600
ccatggtact acgacccttc actaaaatta tcaatcgaag gtggagacgt atttatctac 660
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<210> 284
<211> 1233
<212> DNA
<213> Mycoplasma arginini

<400> 284
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<210> 285
<211> 1233
<212> DNA

<213> Mycoplasma arginini

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gaatcagttc tagttcacga accaggacgc gaaattgact atattacacc agctagacta 120
gatgaattat tattctcagc tatcttagaa agccacgatg ctagaaaaga acacaaacaa 180
ttcgtagcag aattaaaagc aaacgacatc aatgttgttg aattaattga tttagttgct 240
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ggtatcgaag cagatcacga attaatcgtt gacccaatgc caaacctata cttcacacgt 480
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<210> 286
<211> 1233
<212> DNA
<213> Mycoplasma arginini

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<210> 287
<211> 390
<212> DNA
<213> Homo sapiens

<400> 287
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ttgctgcgct ccgtggccgg ggagcaagcg ccaggcaccg ccccctgctc ccgcggcagc 120

tcctggagcg cggacctgga caagtgcatg gactgcgcgt cttgcagggc gcgaccgcac 180
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ggctgcccag ctgtggcgct gatccagtga 390

<210> 288
<211> 1494
<212> DNA
<213> Homo sapiens

<400> 288
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acatactgtg actcctttga ccccccgacc tttcctgccc ttggtacctt cagccgctat 120
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<210> 289
<211> 1494
<212> DNA
<213> Homo sapiens

<400> 289
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gagagtacac gcagtgggcg acggatggag ctgagtatgg ggcccatcca ggctaatcac 180
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gtacccatgg ccagctgtga cttctccatc cgcacctaca cctatgcaga caccccctgat 420
gatttccagt tgcacaactt cagcctccca gaggaagata ccaagctcaa gataccccctg 480
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tcacccactt ggctcaagac caatggagcg gtgaatggga aggggtcact caagggacag 600
cccggagaca tctaccacca gacctgggcc agatactttg tgaagttcct ggatgcctat 660
gctgagcaca agttacagtt ctgggcagtg acagctgaaa atgagccttc tgctgggctg 720
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gcagctaaat atgttcatgg cattgctgta cattggtacc tggactttct ggctccagcc 960
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gcctgtgtgg gctccaagtt ctgggagcag agtgtgcggc taggctcctg ggatcgaggg 1080
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cccatcattg tagacatcac caaggacacg ttttacaaac agcccatgtt ctaccacctt 1260
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ctaaaccgct cctctaagga tgtgcctctt accatcaagg atcctgctgt gggcttcctg 1440
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<210> 290
<211> 1494
<212> DNA
<213> Homo sapiens

<400> 290
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acatactgtg actcctttga ccccccgacc tttcctgccc ttggtacctt cagccgctat 120
gagagtacac gcagtgggcg acggatggag ctgagtatgg ggcccatcca ggctaatcac 180
acgggcacag gcctgctact gaccctgcag ccagaacaga agttccagaa agtgaaggga 240
tttggagggg ccatgacaga tgctgctgct ctcaacatcc ttgccctgtc acccccctgcc 300
caaaatttgc tacttaaatc gtacttctct gaagaaggaa tcggatataa catcatccgg 360
gtacccatgg ccagctgtga cttctccatc cgcacctaca cctatgcaga caccccctgat 420
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cccggagaca tctaccacca gacctgggcc agatactttg tgaagttcct ggatgcctat 660
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ctaaaccgct cctctaagga tgtgcctctt accatcaagg atcctgctgt gggcttcctg 1440
gagacaatct cacctggcta ctccattcac acctacctgt ggcatcgcca gtga 1494

<210> 291
<211> 1494
<212> DNA
<213> Homo sapiens

<400> 291
gcccgccccc gcatccctaa aagcttcggc tacagctcgg tggtgtgtgt ctgcaatgcc 60
acatactgtg actcctttga ccccccgacc tttcctgccc ttggtacctt cagccgctat 120
gagagtacac gcagtgggcg acggatggag ctgagtatgg ggcccatcca ggctaatcac 180
acgggcacag gcctgctact gaccctgcag ccagaacaga agttccagaa agtgaaggga 240
tttggagggg ccatgacaga tgctgctgct ctcaacatcc ttgccctgtc acccccctgcc 300
caaaatttgc tacttaaatc gtacttctct gaagaaggaa tcggatataa catcatccgg 360
gtacccatgg ccagctgtga cttctccatc cgcacctaca cctatgcaga caccccctgat 420
gatttccagt tgcacaactt cagcctccca gaggaagata ccaagctcaa gataccccctg 480
attcaccgag ccctgcagtt ggcccagcgt cccgtttcac tccttgccag cccctggaca 540
tcacccactt ggctcaagac caatggagcg gtgaatggga aggggtcact caagggacag 600
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gctgagcaca agttacagtt ctgggcagtg acagctgaaa atgagccttc tgctgggctg 720
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gcccgtgacc taggtcctac cctcgccaac agtactcacc acaatgtccg cctactcatg 840

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gcagctaaat atgttcatgg cattgctgta cattggtacc tggactttct ggctccagcc 960
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cccatcattg tagacatcac caaggacacg ttttacaaac agcccatgtt ctaccacctt 1260
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ctaaaccgct cctctaagga tgtgcctctt accatcaagg atcctgctgt gggcttcctg 1440
gagacaatct cacctggcta ctccattcac acctacctgt ggcatcgcca gtga 1494

<210> 292
<211> 156
<212> DNA
<213> Homo sapiens

<400> 292
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tgcacggtgc agaagctggc acaccagatc taccagttca cagataagga caaggacaac 120
gtcgccccca ggagcaagat cagcccccag ggctac 156

<210> 293
<211> 156
<212> DNA
<213> Homo sapiens

<400> 293
taccgccaga gcatgaacaa cttccagggc ctccggagct ttggctgccg cttcgggacg 60
tgcacggtgc agaagctggc acaccagatc taccagttca cagataagga caaggacaac 120
gtcgccccca ggagcaagat cagcccccag ggctac 156

<210> 294
<211> 156
<212> DNA
<213> Homo sapiens

<400> 294
taccgccaga gcatgaacaa cttccagggc ctccggagct ttggctgccg cttcgggacg 60
tgcacggtgc agaagctggc acaccagatc taccagttca cagataagga caaggacaac 120
gtcgccccca ggagcaagat cagcccccag ggctac 156

<210> 295
<211> 156
<212> DNA
<213> Homo sapiens

<400> 295
taccgccaga gcatgaacaa cttccagggc ctccggagct ttggctgccg cttcgggacg 60
tgcacggtgc agaagctggc acaccagatc taccagttca cagataagga caaggacaac 120
gtcgccccca ggagcaagat cagcccccag ggctac 156

<210> 296
<211> 438
<212> DNA
<213> Homo sapiens

<400> 296
atgaactcac tggtttcttg gcagctactg cttttcctct gtgccaccca ctttggggag 60
ccattagaaa aggtggcctc tgtggggaat tctagaccca caggccagca gctagaatcc 120
ctgggcctcc tggcccccgg ggagcagagc ctgccgtgca ccgagaggaa gccagctgct 180

actgccaggc tgagccgtcg ggggacctcg ctgtccccgc cccccgagag ctccgggagc 240
ccccagcagc cgggcctgtc cgcccccccac agccgccaga tccccgcacc ccagggcgcg 300
gtgctggtgc agcgggagaa ggacctgccg aactacaact ggaactcctt cggcctgcgc 360
ttcggcaagc gggaggcggc accagggaac cacggcagaa gcgctgggcg gggctggggc 420
gcaggtgcgg ggcagtga 438

<210> 297
<211> 438
<212> DNA
<213> Homo sapiens

<400> 297
atgaactcac tggtttcttg gcagctactg cttttcctct gtgccaccca ctttggggag 60
ccattagaaa aggtggcctc tgtggggaat tctagaccca caggccagca gctagaatcc 120
ctgggcctcc tggccccccgg ggagcagagc ctgccgtgca ccgagaggaa gccagctgct 180
actgccaggc tgagccgtcg ggggacctcg ctgtccccgc cccccgagag ctccgggagc 240
ccccagcagc cgggcctgtc cgcccccccac agccgccaga tccccgcacc ccagggcgcg 300
gtgctggtgc agcgggagaa ggacctgccg aactacaact ggaactcctt cggcctgcgc 360
ttcggcaagc gggaggcggc accagggaac cacggcagaa gcgctgggcg gggctggggc 420
gcaggtgcgg ggcagtga 438

<210> 298
<211> 1041
<212> DNA
<213> Homo sapiens

<400> 298
atggctcggc ctgggcagcg ttggctcggc aagtggcttg tggcgatggt cgtgtgggcg 60
ctgtgccggc tcgccacacc gctggccaag aacctggagc ccgtatcctg gagctccctc 120
aaccccaagt tcctgagtgg gaagggcttg gtgatctatc cgaaaattgg agacaagctg 180
gacatcatct gccccccgagc agaagcaggg cggccctatg agtactacaa gctgtacctg 240
gtgcggcctg agcaggcagc tgcctgtagc acagttctcg accccaacgt gttggtcacc 300
tgcaataggc cagagcagga aatacgcttt accatcaagt tccaggagtt cagccccaac 360
tacatgggcc tggagttcaa gaagcaccat gattactaca ttacctcaac atccaatgga 420
agcctggagg ggctggaaaa ccgggagggc ggtgtgtgcc gcacacgcac catgaagatc 480
atcatgaagg ttgggcaaga tcccaatgct gtgacgcctg agcagctgac taccagcagg 540
cccagcaagg aggcagacaa cactgtcaag atggccacac aggcccctgg tagtcggggc 600
tccctgggtg actctgatgg caagcatgag actgtgaacc aggaagagaa gagtggccca 660
ggtgcaagtg ggggcagcag cgggggaccct gatggcttct tcaactccaa ggtggcattg 720
ttcgcggctg tcggtgccgg ttgcgtcatc ttcctgctca tcatcatctt cctgacggtc 780
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tcgctcagta ccctggccag tcccaagggg ggcagtggca cagcgggcac cgagcccagc 900
gacatcatca ttcccttacg gactacagag aacaactact gcccccacta tgagaaggtg 960
agtggggact acgggcaccc tgtctacatc gtccaagaga tgccgcccca gagcccggcg 1020
aacatctact acaaggtctg a 1041

<210> 299
<211> 1041
<212> DNA
<213> Homo sapiens

<400> 299
atggctcggc ctgggcagcg ttggctcggc aagtggcttg tggcgatggt cgtgtgggcg 60
ctgtgccggc tcgccacacc gctggccaag aacctggagc ccgtatcctg gagctccctc 120
aaccccaagt tcctgagtgg gaagggcttg gtgatctatc cgaaaattgg agacaagctg 180
gacatcatct gccccccgagc agaagcaggg cggccctatg agtactacaa gctgtacctg 240
gtgcggcctg agcaggcagc tgcctgtagc acagttctcg accccaacgt gttggtcacc 300
tgcaataggc cagagcagga aatacgcttt accatcaagt tccaggagtt cagccccaac 360
tacatgggcc tggagttcaa gaagcaccat gattactaca ttacctcaac atccaatgga 420
agcctggagg ggctggaaaa ccgggagggc ggtgtgtgcc gcacacgcac catgaagatc 480
atcatgaagg ttgggcaaga tcccaatgct gtgacgcctg agcagctgac taccagcagg 540

cccagcaagg aggcagacaa cactgtcaag atggccacac aggcccctgg tagtcggggc 600
tccctgggtg actctgatgg caagcatgag actgtgaacc aggaagagaa gagtggccca 660
ggtgcaagtg ggggcagcag cggggaccct gatggcttct tcaactccaa ggtggcattg 720
ttcgcggctg tcggtgccgg ttgcgtcatc ttcctgctca tcatcatctt cctgacggtc 780
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tcgctcagta ccctggccag tcccaagggg ggcagtggca cagcgggcac cgagcccagc 900
gacatcatca ttcccttacg gactacagag aacaactact gcccccacta tgagaaggtg 960
agtggggact acgggcaccc tgtctacatc gtccaagaga tgccgcccca gagcccggcg 1020
aacatctact acaaggtctg a 1041

<210> 300
<211> 390
<212> DNA
<213> Homo sapiens

<400> 300
atggctcggg gctcgctgcg ccggttgctg cggctcctcg tgctggggct ctggctggcg 60
ttgctgcgct ccgtggccgg ggagcaagcg ccaggcaccg ccccctgctc ccgcggcagc 120
tcctggagcg cggacctgga caagtgcatg gactgcgcgt cttgcagggc gcgaccgcac 180
agcgacttct gcctgggctg cgctgcagca cctcctgccc ccttccggct gctttggccc 240
atccttgggg gcgctctgag cctgaccttc gtgctggggc tgctttctgg ctttttggtc 300
tggagacgat gccgcaggag agagaagttc accaccccca tagaggagac cggcggagag 360
ggctgcccag ctgtggcgct gatccagtga 390

<210> 301
<211> 741
<212> DNA
<213> Homo sapiens

<400> 301
atgctgcgtc ggcggggcag ccctggcatg ggtgtgcatg tgggtgcagc cctgggagca 60
ctgtggttct gcctcacagg agccctggag gtccaggtcc ctgaagaccc agtggtggca 120
ctggtgggca ccgatgccac cctgtgctgc tccttctccc ctgagcctgg cttcagcctg 180
gcacagctca acctcatctg gcagctgaca gacaccaaac agctggtgca cagctttgct 240
gaggggccagg accagggcag cgcctatgcc aaccgcacgg ccctcttcct ggacctgctg 300
gcacagggca acgcatccct gaggctgcag cgcgtgcgtg tggcggacga gggcagcttc 360
acctgcttcg tgagcatccg ggatttcggc agcgctgccg tcagcctgca ggtggccgct 420
ccctactcga agcccagcat gaccctggag cccaacaagg acctgcggcc aggggacacg 480
gtgaccatca cgtgctccag ctaccggggc taccctgagg ctgaggtgtt ctggcaggat 540
gggcagggtg tgcccctgac tggcaacgtg accacgtcgc agatggccaa cgagcagggc 600
ttgtttgatg tgcacagcgt cctgcgggtg gtgctgggtg cgaatggcac ctacagctgc 660
ctggtgcgca accccgtgct gcagcaggat gcgcacggct ctgtcaccat cacagggcag 720
cctatgacat tccccccaga g 741

<210> 302
<211> 1041
<212> DNA
<213> Homo sapiens

<400> 302
atggctcggc ctgggcagcg ttggctcggc aagtggcttg tggcgatggt cgtgtgggcg 60
ctgtgccggc tcgccacacc gctggccaag aacctggagc ccgtatcctg gagctccctc 120
aacccccaagt tcctgagtgg gaagggcttg gtgatctatc cgaaaattgg agacaagctg 180
gacatcatct gccccgagc agaagcaggg cggccctatg agtactacaa gctgtacctg 240
gtgcggcctg agcaggcagc tgcctgtagc acagttctcg accccaacgt gttggtcacc 300
tgcaataggc cagagcagga aatacgcttt accatcaagt tccaggagtt cagccccaac 360
tacatgggcc tggagttcaa gaagcaccat gattactaca ttacctcaac atccaatgga 420
agcctggagg ggctggaaaa ccgggagggc ggtgtgtgcc gcacacgcac catgaagatc 480
atcatgaagg ttgggcaaga tcccaatgct gtgacgcctg agcagctgac taccagcagg 540
cccagcaagg aggcagacaa cactgtcaag atggccacac aggcccctgg tagtcggggc 600
tccctgggtg actctgatgg caagcatgag actgtgaacc aggaagagaa gagtggccca 660

ggtgcaagtg ggggcagcag cggggaccct gatggcttct tcaactccaa ggtggcattg 720
ttcgcggctg tcggtgccgg ttgcgtcatc ttcctgctca tcatcatctt cctgacggtc 780
ctactactga agctacgcaa gcggcaccgc aagcacacac agcagcgggc ggctgccctc 840
tcgctcagta ccctggccag tcccaagggg ggcagtggca cagcgggcac cgagcccagc 900
gacatcatca ttcccttacg gactacagag aacaactact gcccccacta tgagaaggtg 960
agtggggact acgggcaccc tgtctacatc gtccaagaga tgccgcccca gagcccggcg 1020
aacatctact acaaggtctg a 1041

<210> 303
<211> 1041
<212> DNA
<213> Homo sapiens

<400> 303
atggctcggc ctgggcagcg ttggctcggc aagtggcttg tggcgatggt cgtgtgggcg 60
ctgtgccggc tcgccacacc gctggccaag aacctggagc ccgtatcctg gagctccctc 120
aaccccaagt tcctgagtgg gaagggcttg gtgatctatc cgaaaattgg agacaagctg 180
gacatcatct gcccccgagc agaagcaggg cggccctatg agtactacaa gctgtacctg 240
gtgcggcctg agcaggcagc tgcctgtagc acagttctcg accccaacgt gttggtcacc 300
tgcaataggc cagagcagga aatacgcttt accatcaagt tccaggagtt cagccccaac 360
tacatgggcc tggagttcaa gaagcaccat gattactaca ttacctcaac atccaatgga 420
agcctggagg ggctggaaaa ccgggagggc ggtgtgtgcc gcacacgcac catgaagatc 480
atcatgaagg ttgggcaaga tcccaatgct gtgacgcctg agcagctgac taccagcagg 540
cccagcaagg aggcagacaa cactgtcaag atggccacac aggccccctgg tagtcggggc 600
tccctgggtg actctgatgg caagcatgag actgtgaacc aggaagagaa gagtggccca 660
ggtgcaagtg ggggcagcag cggggaccct gatggcttct tcaactccaa ggtggcattg 720
ttcgcggctg tcggtgccgg ttgcgtcatc ttcctgctca tcatcatctt cctgacggtc 780
ctactactga agctacgcaa gcggcaccgc aagcacacac agcagcgggc ggctgccctc 840
tcgctcagta ccctggccag tcccaagggg ggcagtggca cagcgggcac cgagcccagc 900
gacatcatca ttcccttacg gactacagag aacaactact gcccccacta tgagaaggtg 960
agtggggact acgggcaccc tgtctacatc gtccaagaga tgccgcccca gagcccggcg 1020
aacatctact acaaggtctg a 1041

<210> 304
<211> 960
<212> DNA
<213> Pan troglodytes

<220>
<221> misc_difference
<222> (88) to (90)
<223> Natural stop codon replaced by Arg

<400> 304
atgcctgtgt tttcattgca gaatgatgag gtggagtttg tccgaactgg ctatgggaag 60
gatatagtaa aagttctcca tattcagcga gatggaaaat atcacagcat taaagaggtg 120
gcaacttcag tgcaacttac tctaagttcc aaaaaagatt acctgcatgg agataattca 180
gacatcatcc ctacagacac catcaagaac acagttcatg tcttggcaaa gtttaaagaa 240
aatgaaccag caaacataga tggggctatg gaaaaagcat tttgtttctt tttacaaatc 300
aaaagcatag aagcctttgg tgtgaatatt tgtgagcatt ttctttcttc ttttaaccat 360
gtaatccgag ctcaagtcta tgtggaagaa atcccttgga agcatcttga aaagaatgga 420
gttaagcatg tccatgcatt tattcacact cccactggaa cacacttcgg tgaagttgaa 480
cagctgagaa gtggacccca agtcattcat tctggaatca aagacctcaa gctcttgaaa 540
acaacacagt ctggatttga aggtttcatc aaggaccagt tcactaccct ccctgaggtt 600
tttaaatgca tgatgagaac gttacctcag tcttctttcc ctttatttca agtgctctcc 660
atgggcagca gcctgatgga caccattcgg gaccttgtca tggagaaatc tgctgggccc 720
tatgacaaag atgaatactc gcccctctgtg cagaagaccc tctgtgatat ccaggtgctc 780
tccctgagcc gagttcctgc gatagaagat atggaaatca gcctgccaaa cattcactac 840
ttcaacatag acatgtccaa aatgggtctg atcaacaagg aagaggtctt gctgccatta 900
gacaatccat atggaaaaat tactggtaca gtcaagagga agttgtcttc aagactgtga 960

<210> 305
<211> 960
<212> DNA
<213> Pan troglodytes

<220>
<221> misc_difference
<222> (88) to (90)
<223> Natural stop codon replaced by Arg

<400> 305
atgcctgtgt tttcattgca gaatgatgag gtggagtttg tccgaactgg ctatgggaag 60
gatatagtaa aagttctcca tattcagcga gatggaaaat atcacagcat taaagaggtg 120
gcaacttcag tgcaacttac tctaagttcc aaaaaagatt acctgcatgg agataattca 180
gacatcatcc ctacagacac catcaagaac acagttcatg tcttggcaaa gtttaaagaa 240
aatgaaccag caaacataga tggggctatg gaaaaagcat tttgtttctt tttacaaatc 300
aaaagcatag aagcctttgg tgtgaatatt tgtgagcatt ttctttcttc ttttaaccat 360
gtaatccgag ctcaagtcta tgtggaagaa atcccttgga agcatcttga aaagaatgga 420
gttaagcatg tccatgcatt tattcacact cccactggaa cacacttcgg tgaagttgaa 480
cagctgagaa gtggacccca agtcattcat tctggaatca aagacctcaa gctcttgaaa 540
acaacacagt ctggatttga aggtttcatc aaggaccagt tcactaccct ccctgaggtt 600
tttaaatgca tgatgagaac gttacctcag tcttctttcc ctttatttca agtgctctcc 660
atgggcagca gcctgatgga caccattcgg gaccttgtca tggagaaatc tgctgggccc 720
tatgacaaag atgaatactc gccctctgtg cagaagaccc tctgtgatat ccaggtgctc 780
tccctgagcc gagttcctgc gatagaagat atggaaatca gcctgccaaa cattcactac 840
ttcaacatag acatgtccaa aatgggtctg atcaacaagg aagaggtctt gctgccatta 900
gacaatccat atggaaaaat tactggtaca gtcaagagga agttgtcttc aagactgtga 960

<210> 306
<211> 1186
<212> DNA
<213> Homo sapiens

<220>
<221> misc_difference
<222> (310) to (312)
<223> Natural stop codon replaced by Arg

<220>
<221> misc_difference
<222> (829) to (831)
<223> Natural stop codon replaced by Arg

<220>
<221> misc_feature
<222> (1186)
<223> n equals a,t,g, or c

<400> 306
atggagaaat tcatttggta cctggtgcat ttgtacactg aaatgaccaa gtcttctccc 60
tcctgccgcc tcgtggctag ttgtcagacc gtggcaaaag aactgggggg gaacagcctc 120
ggttatggtc caggccatta tctgctcttt gggtgcaggg atgcttttgg gtgtcccatg 180
ccaggcttgt tccacctgct ccaggatcag atgattggtt cccttcatac agactcattg 240
ccaaatgatg aggtggagtt tgtccgaact ggctatggga aggaaatggt aaaagttctc 300
catattcagc gagatggaaa atatcacagc attaaagagg tggcaacttc agtgcaactt 360
actctaagtt ccaaaaaaga ttacctgcat ggagataatt cagacatcat ccctacagac 420
accatcaaga acacagttca tgtcttggca aagtttaaag aaaatgaccc agcaaacata 480
gatggggcta tggaaaaagc attttgtttc tttttacaaa tcaaaagcat agaagccttt 540
ggtgtgaata tttgtgagca ttttctttct tcttttaacc atgtaatccg agctcaagtc 600
tacatggaag aaatcccttg gaagcatctt ggaaagaatg gagttaagca tgtccatgca 660
tttattcaca ctcccactgg aacacacttc tgtgaagttg aacagctgag aagtggaccc 720

caagtcattc attctggaat caaagacctc aaggtcttga aaacaacaca gtctggattt 780
gaaggtttca tcaaggacca gttcactacc ctccctgagg tgaaggaccg atgctttgcc 840
acccaagtgt actgcaagtg gcgctaccac cagtgcaggg atgtggactt caaggctacc 900
tgggacacca ttcgggacct tgtcatggag aaatctgctg ggccctatga caaaggtgaa 960
tacttgacct ctgtgcagaa gaccctctgt gatatccagg tgctctccct gagccgagtt 1020
cctgcgatag aagatatgga aatcagcctg ccaaacattc actacttcaa catagacatg 1080
tccaaaatgg gtctgatcaa caaggaagag gtcttgctgc cattagacaa tccatatgga 1140
aaaattactg gtacagtcaa gaggaagttg tcttcaagac tgtgan 1186

<210> 307
<211> 1186
<212> DNA
<213> Homo sapiens

<220>
<221> misc_difference
<222> (310) to (312)
<223> Natural stop codon replaced by Arg

<220>
<221> misc_difference
<222> (829) to (831)
<223> Natural stop codon replaced by Arg

<220>
<221> misc_feature
<222> (1186)
<223> n equals a,t,g, or c

<400> 307
atggagaaat tcatttggta cctggtgcat ttgtacactg aaatgaccaa gtcttctccc 60
tcctgccgcc tcgtggctag ttgtcagacc gtggcaaaag aactgggggg gaacagcctc 120
ggttatggtc caggccatta tctgctcttt gggtgcaggg atgcttttgg gtgtcccatg 180
ccaggcttgt tccacctgct ccaggatcag atgattggtt cccttcatac agactcattg 240
ccaaatgatg aggtggagtt tgtccgaact ggctatggga aggaaatggt aaaagttctc 300
catattcagc gagatggaaa atatcacagc attaaagagg tggcaacttc agtgcaactt 360
actctaagtt ccaaaaaaga ttacctgcat ggagataatt cagacatcat ccctacagac 420
accatcaaga acacagttca tgtcttggca aagtttaaag aaaatgaccc agcaaacata 480
gatggggcta tggaaaaagc attttgtttc tttttacaaa tcaaaagcat agaagccttt 540
ggtgtgaata tttgtgagca ttttctttct tcttttaacc atgtaatccg agctcaagtc 600
tacatggaag aaatcccttg gaagcatctt ggaaagaatg gagttaagca tgtccatgca 660
tttattcaca ctcccactgg aacacacttc tgtgaagttg aacagctgag aagtggaccc 720
caagtcattc attctggaat caaagacctc aaggtcttga aaacaacaca gtctggattt 780
gaaggtttca tcaaggacca gttcactacc ctccctgagg tgaaggaccg atgctttgcc 840
acccaagtgt actgcaagtg gcgctaccac cagtgcaggg atgtggactt caaggctacc 900
tgggacacca ttcgggacct tgtcatggag aaatctgctg ggccctatga caaaggtgaa 960
tacttgacct ctgtgcagaa gaccctctgt gatatccagg tgctctccct gagccgagtt 1020
cctgcgatag aagatatgga aatcagcctg ccaaacattc actacttcaa catagacatg 1080
tccaaaatgg gtctgatcaa caaggaagag gtcttgctgc cattagacaa tccatatgga 1140
aaaattactg gtacagtcaa gaggaagttg tcttcaagac tgtgan 1186

<210> 308
<211> 927
<212> DNA
<213> Papio hamadryas

<220>
<221> misc_feature
<222> (927)
<223> n equals a,t,g, or c

<400> 308
atggccgact accataacaa ctataaaaag aatgatgaat tggagtttgt ccgaactggc 60
tatgggaagg atatggtaaa agttctccat attcagcgag atggaaaata tcacagcatt 120
aaagaggtgg caacttcagt gcaacttact ctgagttcca aaaaagatta cctgcatgga 180
gataattcag atatcatccc tacagacacc atcaagaaca cagttcatgt cttggcaaag 240
tttaagggaa tcaaaagcat agaagccttt ggtgtgaata tttgtgagta ttttctttct 300
tcttttaacc atgtaatccg agctcaagtc tacgtggaag aaatcccttg gaagcgtctt 360
gaaaagaatg gagttaagca tgtccatgca tttattcaca ctcccactgg aacacacttc 420
tgtgaagttg aacaactgag aagtggaccc cccgtcatta cttctggaat caaagacctc 480
aaggtcttga aaacaacaca gtctggattt gaaggtttca tcaaggacca gttcaccacc 540
ctccctgagg tgaaggaccg atgctttgcc acccaagtgt actgcaagtg gcgctaccac 600
cagtgcaggg atgtggactt cgaggctacc tggggcacca ttcgggacct tgtcctggag 660
aaatttgctg ggccctatga caaaggcgag tactcaccct ctgtgcagaa gaccctctat 720
gatatccagg tgctctccct gagccgagtt cctgagatag aagatatgga aatcagcctg 780
ccaaacattc actacttcaa tatagacatg tccaaaatgg gtctgatcaa caaggaagag 840
gtcttgctgc cattagacaa tccatatgga aaaattactg gtacagtcaa gaggaagttg 900
tcttcaagac tgtgacattg tggccan 927

<210> 309
<211> 927
<212> DNA
<213> Papio hamadryas

<220>
<221> misc_feature
<222> (927)
<223> n equals a,t,g, or c

<400> 309
atggccgact accataacaa ctataaaaag aatgatgaat tggagtttgt ccgaactggc 60
tatgggaagg atatggtaaa agttctccat attcagcgag atggaaaata tcacagcatt 120
aaagaggtgg caacttcagt gcaacttact ctgagttcca aaaaagatta cctgcatgga 180
gataattcag atatcatccc tacagacacc atcaagaaca cagttcatgt cttggcaaag 240
tttaagggaa tcaaaagcat agaagccttt ggtgtgaata tttgtgagta ttttctttct 300
tcttttaacc atgtaatccg agctcaagtc tacgtggaag aaatcccttg gaagcgtctt 360
gaaaagaatg gagttaagca tgtccatgca tttattcaca ctcccactgg aacacacttc 420
tgtgaagttg aacaactgag aagtggaccc cccgtcatta cttctggaat caaagacctc 480
aaggtcttga aaacaacaca gtctggattt gaaggtttca tcaaggacca gttcaccacc 540
ctccctgagg tgaaggaccg atgctttgcc acccaagtgt actgcaagtg gcgctaccac 600
cagtgcaggg atgtggactt cgaggctacc tggggcacca ttcgggacct tgtcctggag 660
aaatttgctg ggccctatga caaaggcgag tactcaccct ctgtgcagaa gaccctctat 720
gatatccagg tgctctccct gagccgagtt cctgagatag aagatatgga aatcagcctg 780
ccaaacattc actacttcaa tatagacatg tccaaaatgg gtctgatcaa caaggaagag 840
gtcttgctgc cattagacaa tccatatgga aaaattactg gtacagtcaa gaggaagttg 900
tcttcaagac tgtgacattg tggccan 927

<210> 310
<211> 1186
<212> DNA
<213> Homo sapiens

<220>
<221> misc_difference
<222> (310) to (312)
<223> Natural stop codon replaced by Arg

<220>
<221> misc_difference
<222> (829) to (831)
<223> Natural stop codon replaced by Arg

<220>
<221> misc_feature
<222> (1186)
<223> n equals a,t,g, or c

<400> 310
atggagaaat tcatttggta cctggtgcat ttgtacactg aaatgaccaa gtcttctccc 60
tcctgccgcc tcgtggctag ttgtcagacc gtggcaaaag aactgggggg gaacagcctc 120
ggttatggtc caggccatta tctgctcttt gggtgcaggg atgcttttgg gtgtcccatg 180
ccaggcttgt tccacctgct ccaggatcag atgattggtt cccttcatac agactcattg 240
ccaaatgatg aggtggagtt tgtccgaact ggctatggga aggaaatggt aaaagttctc 300
catattcagc gagatggaaa atatcacagc attaaagagg tggcaacttc agtgcaactt 360
actctaagtt ccaaaaaaga ttacctgcat ggagataatt cagacatcat ccctacagac 420
accatcaaga acacagttca tgtcttggca aagtttaaag aaaatgaccc agcaaacata 480
gatggggcta tggaaaaagc attttgtttc tttttacaaa tcaaaagcat agaagccttt 540
ggtgtgaata tttgtgagca ttttctttct tcttttaacc atgtaatccg agctcaagtc 600
tacatggaag aaatcccttg gaagcatctt ggaaagaatg gagttaagca tgtccatgca 660
tttattcaca ctcccactgg aacacacttc tgtgaagttg aacagctgag aagtggaccc 720
caagtcattc attctggaat caaagacctc aaggtcttga aaacaacaca gtctggattt 780
gaaggtttca tcaaggacca gttcactacc ctccctgagg tgaaggaccg atgctttgcc 840
acccaagtgt actgcaagtg gcgctaccac cagtgcaggg atgtggactt caaggctacc 900
tgggacacca ttcgggacct tgtcatggag aaatctgctg ggccctatga caaaggtgaa 960
tacttgacct ctgtgcagaa gaccctctgt gatatccagg tgctctccct gagccgagtt 1020
cctgcgatag aagatatgga aatcagcctg ccaaacattc actacttcaa catagacatg 1080
tccaaaatgg gtctgatcaa caaggaagag gtcttgctgc cattagacaa tccatatgga 1140
aaaattactg gtacagtcaa gaggaagttg tcttcaagac tgtgan 1186

<210> 311
<211> 960
<212> DNA
<213> Pan troglodytes

<220>
<221> misc_difference
<222> (88) to (90)
<223> Natural stop codon replaced by Arg

<400> 311
atgcctgtgt tttcattgca gaatgatgag gtggagtttg tccgaactgg ctatgggaag 60
gatatagtaa aagttctcca tattcagcga gatggaaaat atcacagcat taaagaggtg 120
gcaacttcag tgcaacttac tctaagttcc aaaaaagatt acctgcatgg agataattca 180
gacatcatcc ctacagacac catcaagaac acagttcatg tcttggcaaa gtttaaagaa 240
aatgaaccag caaacataga tggggctatg gaaaaagcat tttgtttctt tttacaaatc 300
aaaagcatag aagcctttgg tgtgaatatt tgtgagcatt ttctttcttc ttttaaccat 360
gtaatccgag ctcaagtcta tgtggaagaa atcccttgga agcatcttga aaagaatgga 420
gttaagcatg tccatgcatt tattcacact cccactggaa cacacttcgg tgaagttgaa 480
cagctgagaa gtggacccca agtcattcat tctggaatca aagacctcaa gctcttgaaa 540
acaacacagt ctggatttga aggtttcatc aaggaccagt tcactaccct ccctgaggtt 600
tttaaatgca tgatgagaac gttacctcag tcttctttcc ctttatttca agtgctctcc 660
atgggcagca gcctgatgga caccattcgg gaccttgtca tggagaaatc tgctgggccc 720
tatgacaaag atgaatactc gccctctgtg cagaagaccc tctgtgatat ccaggtgctc 780
tccctgagcc gagttcctgc gatagaagat atggaaatca gcctgccaaa cattcactac 840
ttcaacatag acatgtccaa aatgggtctg atcaacaagg aagaggtctt gctgccatta 900
gacaatccat atggaaaaat tactggtaca gtcaagagga agttgtcttc aagactgtga 960

<210> 312
<211> 927
<212> DNA
<213> Papio hamadryas

<220>

<221> misc_feature
<222> (927)
<223> n equals a,t,g, or c

<400> 312
atggccgact accataacaa ctataaaaag aatgatgaat tggagtttgt ccgaactggc 60
tatgggaagg atatggtaaa agttctccat attcagcgag atggaaaata tcacagcatt 120
aaagaggtgg caacttcagt gcaacttact ctgagttcca aaaaagatta cctgcatgga 180
gataattcag atatcatccc tacagacacc atcaagaaca cagttcatgt cttggcaaag 240
tttaagggaa tcaaaagcat agaagccttt ggtgtgaata tttgtgagta ttttctttct 300
tcttttaacc atgtaatccg agctcaagtc tacgtggaag aaatcccttg gaagcgtctt 360
gaaaagaatg gagttaagca tgtccatgca tttattcaca ctcccactgg aacacacttc 420
tgtgaagttg aacaactgag aagtggaccc cccgtcatta cttctggaat caaagacctc 480
aaggtcttga aaacaacaca gtctggattt gaaggtttca tcaaggacca gttcaccacc 540
ctccctgagg tgaaggaccg atgctttgcc acccaagtgt actgcaagtg gcgctaccac 600
cagtgcaggg atgtggactt cgaggctacc tggggcacca ttcgggacct tgtcctggag 660
aaatttgctg ggccctatga caaaggcgag tactcaccct ctgtgcagaa gaccctctat 720
gatatccagg tgctctccct gagccgagtt cctgagatag aagatatgga aatcagcctg 780
ccaaacattc actacttcaa tatagacatg tccaaaatgg gtctgatcaa caaggaagag 840
gtcttgctgc cattagacaa tccatatgga aaaattactg gtacagtcaa gaggaagttg 900
tcttcaagac tgtgacattg tggccan 927

<210> 313
<211> 805
<212> PRT
<213> Homo sapiens

<400> 313
Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
1 5 10 15

Leu Glu His Thr His Arg Arg Gly Ser Leu Asp Lys Arg Asp Ala His
20 25 30

Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe
35 40 45

Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro
50 55 60

Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys
65 70 75 80

Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His
85 90 95

Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr
100 105 110

Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn
115 120 125

Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu
130 135 140

Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu
145 150 155 160

Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro
165 170 175

Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala
180 185 190

Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu
195 200 205

Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys
210 215 220

Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe
225 230 235 240

Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu
245 250 255

Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr
260 265 270

Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp
275 280 285

Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu
290 295 300

Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala
305 310 315 320

Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala
325 330 335

Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys
340 345 350

Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro
355 360 365

Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr
370 375 380

Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala
385 390 395 400

Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu
405 410 415

Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe
420 425 430

Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser
435 440 445

Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser
450 455 460

Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp
465 470 475 480

Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr
485 490 495

Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn

500 505 510

Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro
515 520 525

Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr
530 535 540

Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu
545 550 555 560

Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val
565 570 575

Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp
580 585 590

Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser
595 600 605

Gln Ala Ala Leu Gly Leu Phe Pro Thr Ile Pro Leu Ser Arg Leu Phe
610 615 620

Asp Asn Ala Met Leu Arg Ala His Arg Leu His Gln Leu Ala Phe Asp
625 630 635 640

Thr Tyr Gln Glu Phe Glu Glu Ala Tyr Ile Pro Lys Glu Gln Lys Tyr
645 650 655

Ser Phe Leu Gln Asn Pro Gln Thr Ser Leu Cys Phe Ser Glu Ser Ile
660 665 670

Pro Thr Pro Ser Asn Arg Glu Glu Thr Gln Gln Lys Ser Asn Leu Glu
675 680 685

Leu Leu Arg Ile Ser Leu Leu Leu Ile Gln Ser Trp Leu Glu Pro Val
690 695 700

Gln Phe Leu Arg Ser Val Phe Ala Asn Ser Leu Val Tyr Gly Ala Ser
705 710 715 720

Asp Ser Asn Val Tyr Asp Leu Leu Lys Asp Leu Glu Glu Gly Ile Gln
725 730 735

Thr Leu Met Gly Arg Leu Glu Asp Gly Ser Pro Arg Thr Gly Gln Ile
740 745 750

Phe Lys Gln Thr Tyr Ser Lys Phe Asp Thr Asn Ser His Asn Asp Asp
755 760 765

Ala Leu Leu Lys Asn Tyr Gly Leu Leu Tyr Cys Phe Arg Lys Asp Met
770 775 780

Asp Lys Val Glu Thr Phe Leu Arg Ile Val Gln Cys Arg Ser Val Glu
785 790 795 800

Gly Ser Cys Gly Phe
805

<210> 314
<211> 795
<212> PRT
<213> Homo sapiens

<400> 314
Met Leu Leu Gln Ala Phe Leu Phe Leu Leu Ala Gly Phe Ala Ala Lys
1 5 10 15

Ile Ser Ala Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp
20 25 30

Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln
35 40 45

Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu
50 55 60

Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn
65 70 75 80

Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val
85 90 95

Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys
100 105 110

Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn
115 120 125

Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr
130 135 140

Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu
145 150 155 160

Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe
165 170 175

Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp
180 185 190

Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly
195 200 205

Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys
210 215 220

Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln
225 230 235 240

Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp
245 250 255

Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys
260 265 270

Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp
275 280 285

Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu

290 295 300

Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp
305 310 315 320

Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys
325 330 335

Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu
340 345 350

Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu
355 360 365

Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp
370 375 380

Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val
385 390 395 400

Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln
405 410 415

Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys
420 425 430

Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn
435 440 445

Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg
450 455 460

Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys
465 470 475 480

Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys
485 490 495

Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val
500 505 510

Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe
515 520 525

His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys
530 535 540

Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys
545 550 555 560

Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys
565 570 575

Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys
580 585 590

Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu Phe Pro Thr Ile
595 600 605

Pro Leu Ser Arg Leu Phe Asp Asn Ala Met Leu Arg Ala His Arg Leu
610 615 620

His Gln Leu Ala Phe Asp Thr Tyr Gln Glu Phe Glu Glu Ala Tyr Ile
625 630 635 640

Pro Lys Glu Gln Lys Tyr Ser Phe Leu Gln Asn Pro Gln Thr Ser Leu
645 650 655

Cys Phe Ser Glu Ser Ile Pro Thr Pro Ser Asn Arg Glu Glu Thr Gln
660 665 670

Gln Lys Ser Asn Leu Glu Leu Leu Arg Ile Ser Leu Leu Leu Ile Gln
675 680 685

Ser Trp Leu Glu Pro Val Gln Phe Leu Arg Ser Val Phe Ala Asn Ser
690 695 700

Leu Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr Asp Leu Leu Lys Asp
705 710 715 720

Leu Glu Glu Gly Ile Gln Thr Leu Met Gly Arg Leu Glu Asp Gly Ser
725 730 735

Pro Arg Thr Gly Gln Ile Phe Lys Gln Thr Tyr Ser Lys Phe Asp Thr
740 745 750

Asn Ser His Asn Asp Asp Ala Leu Leu Lys Asn Tyr Gly Leu Leu Tyr
755 760 765

Cys Phe Arg Lys Asp Met Asp Lys Val Glu Thr Phe Leu Arg Ile Val
770 775 780

Gln Cys Arg Ser Val Glu Gly Ser Cys Gly Phe
785 790 795

<210> 315
<211> 793
<212> PRT
<213> Homo sapiens

<400> 315
Met Phe Lys Ser Val Val Tyr Ser Ile Leu Ala Ala Ser Leu Ala Asn
1 5 10 15

Ala Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly
20 25 30

Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu
35 40 45

Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr
50 55 60

Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp
65 70 75 80

Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr
85 90 95

Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu

100 105 110

Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn
115 120 125

Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe
130 135 140

His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala
145 150 155 160

Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys
165 170 175

Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala
180 185 190

Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala
195 200 205

Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly
210 215 220

Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe
225 230 235 240

Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr
245 250 255

Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp
260 265 270

Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile
275 280 285

Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser
290 295 300

His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro
305 310 315 320

Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr
325 330 335

Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala
340 345 350

Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys
355 360 365

Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His
370 375 380

Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu
385 390 395 400

Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly
405 410 415

Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val
420 425 430

Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly
435 440 445

Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro
450 455 460

Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu
465 470 475 480

His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu
485 490 495

Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu
500 505 510

Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala
515 520 525

Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr
530 535 540

Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln
545 550 555 560

Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys
565 570 575

Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu
580 585 590

Val Ala Ala Ser Gln Ala Ala Leu Gly Leu Phe Pro Thr Ile Pro Leu
595 600 605

Ser Arg Leu Phe Asp Asn Ala Met Leu Arg Ala His Arg Leu His Gln
610 615 620

Leu Ala Phe Asp Thr Tyr Gln Glu Phe Glu Glu Ala Tyr Ile Pro Lys
625 630 635 640

Glu Gln Lys Tyr Ser Phe Leu Gln Asn Pro Gln Thr Ser Leu Cys Phe
645 650 655

Ser Glu Ser Ile Pro Thr Pro Ser Asn Arg Glu Glu Thr Gln Gln Lys
660 665 670

Ser Asn Leu Glu Leu Leu Arg Ile Ser Leu Leu Leu Ile Gln Ser Trp
675 680 685

Leu Glu Pro Val Gln Phe Leu Arg Ser Val Phe Ala Asn Ser Leu Val
690 695 700

Tyr Gly Ala Ser Asp Ser Asn Val Tyr Asp Leu Leu Lys Asp Leu Glu
705 710 715 720

Glu Gly Ile Gln Thr Leu Met Gly Arg Leu Glu Asp Gly Ser Pro Arg
725 730 735

Thr Gly Gln Ile Phe Lys Gln Thr Tyr Ser Lys Phe Asp Thr Asn Ser
740 745 750

His Asn Asp Asp Ala Leu Leu Lys Asn Tyr Gly Leu Leu Tyr Cys Phe
755 760 765

Arg Lys Asp Met Asp Lys Val Glu Thr Phe Leu Arg Ile Val Gln Cys
770 775 780

Arg Ser Val Glu Gly Ser Cys Gly Phe
785 790

<210> 316
<211> 800
<212> PRT
<213> Homo sapiens

<400> 316
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Gly Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Phe Pro Thr Ile Pro Leu Ser Arg Leu Phe Asp Asn Ala Met Leu
610 615 620

Arg Ala His Arg Leu His Gln Leu Ala Phe Asp Thr Tyr Gln Glu Phe
625 630 635 640

Glu Glu Ala Tyr Ile Pro Lys Glu Gln Lys Tyr Ser Phe Leu Gln Asn
645 650 655

Pro Gln Thr Ser Leu Cys Phe Ser Glu Ser Ile Pro Thr Pro Ser Asn
660 665 670

Arg Glu Glu Thr Gln Gln Lys Ser Asn Leu Glu Leu Leu Arg Ile Ser
675 680 685

Leu Leu Leu Ile Gln Ser Trp Leu Glu Pro Val Gln Phe Leu Arg Ser
690 695 700

Val Phe Ala Asn Ser Leu Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr
705 710 715 720

Asp Leu Leu Lys Asp Leu Glu Glu Gly Ile Gln Thr Leu Met Gly Arg
725 730 735

Leu Glu Asp Gly Ser Pro Arg Thr Gly Gln Ile Phe Lys Gln Thr Tyr
740 745 750

Ser Lys Phe Asp Thr Asn Ser His Asn Asp Asp Ala Leu Leu Lys Asn
755 760 765

Tyr Gly Leu Leu Tyr Cys Phe Arg Lys Asp Met Asp Lys Val Glu Thr
770 775 780

Phe Leu Arg Ile Val Gln Cys Arg Ser Val Glu Gly Ser Cys Gly Phe
785 790 795 800

<210> 317
<211> 774
<212> PRT
<213> Homo sapiens

<400> 317
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Gly Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu
610 615 620

Met Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys
625 630 635 640

Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe
645 650 655

Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile
660 665 670

Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
675 680 685

Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu

690 695 700

Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met
705 710 715 720

Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
725 730 735

Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
740 745 750

Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
755 760 765

Ser Leu Arg Ser Lys Glu
770

<210> 318
<211> 728
<212> PRT
<213> Homo sapiens

<400> 318
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg His Ser Asp Pro Ala Arg Arg Gly
20 25 30

Glu Leu Ser Val Cys Asp Ser Ile Ser Glu Trp Val Thr Ala Ala Asp
35 40 45

Lys Lys Thr Ala Val Asp Met Ser Gly Gly Thr Val Thr Val Leu Glu
50 55 60

Lys Val Pro Val Ser Lys Gly Gln Leu Lys Gln Tyr Phe Tyr Glu Thr
65 70 75 80

Lys Cys Asn Pro Met Gly Tyr Thr Lys Glu Gly Cys Arg Gly Ile Asp
85 90 95

Lys Arg His Trp Asn Ser Gln Cys Arg Thr Thr Gln Ser Tyr Val Arg
100 105 110

Ala Leu Thr Met Asp Ser Lys Lys Arg Ile Gly Trp Arg Phe Ile Arg
115 120 125

Ile Asp Thr Ser Cys Val Cys Thr Leu Thr Ile Lys Arg Gly Arg Asp
130 135 140

Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu
145 150 155 160

Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln
165 170 175

Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe
180 185 190

Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser
195 200 205

Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg
210 215 220

Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu
225 230 235 240

Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro
245 250 255

Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp
260 265 270

Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg
275 280 285

His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr
290 295 300

Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys
305 310 315 320

Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser
325 330 335

Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg
340 345 350

Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys
355 360 365

Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val
370 375 380

His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg
385 390 395 400

Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser
405 410 415

Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys
420 425 430

Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu
435 440 445

Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu
450 455 460

Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg
465 470 475 480

His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr
485 490 495

Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys
500 505 510

Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln

515 520 525

Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr
530 535 540

Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln
545 550 555 560

Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val
565 570 575

Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala
580 585 590

Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu
595 600 605

Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu
610 615 620

Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr
625 630 635 640

Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile
645 650 655

Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu
660 665 670

Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys
675 680 685

Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala
690 695 700

Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala
705 710 715 720

Ala Ser Gln Ala Ala Leu Gly Leu
725

<210> 319
<211> 669
<212> PRT
<213> Homo sapiens

<400> 319
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Gly Ser Leu Asp Lys Arg His Gly Glu Gly Thr Phe Thr Ser
20 25 30

Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala
35 40 45

Trp Leu Val Lys Gly Arg His Gly Glu Gly Thr Phe Thr Ser Asp Val
50 55 60

Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu

65 70 75 80

Val Lys Gly Arg Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys
85 90 95

Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala
100 105 110

Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn
115 120 125

Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu
130 135 140

Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr
145 150 155 160

Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala
165 170 175

Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp
180 185 190

Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys
195 200 205

Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr
210 215 220

Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe
225 230 235 240

Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala
245 250 255

Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu
260 265 270

Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln
275 280 285

Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser
290 295 300

Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr
305 310 315 320

Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu
325 330 335

Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln
340 345 350

Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu
355 360 365

Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala
370 375 380

Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys
385 390 395 400

Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr
405 410 415

Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg
420 425 430

Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala
435 440 445

Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu
450 455 460

Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu
465 470 475 480

Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr
485 490 495

Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg
500 505 510

Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys
515 520 525

Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu
530 535 540

Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys
545 550 555 560

Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu
565 570 575

Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr
580 585 590

Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys
595 600 605

Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr
610 615 620

Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu
625 630 635 640

Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly
645 650 655

Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
660 665

<210> 320
<211> 638
<212> PRT
<213> Homo sapiens

<400> 320
Met Lys Val Ser Val Ala Ala Leu Ser Cys Leu Met Leu Val Thr Ala

396 394

```
  1               5                  10                  15

Leu Gly Ser Gln Ala Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe
             20                  25                  30

Gly Arg Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys
         35                  40                  45

Val Leu Arg Arg His Asp Ala His Lys Ser Glu Val Ala His Arg Phe
     50                  55                  60

Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe
 65                  70                  75                  80

Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val
                 85                  90                  95

Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala
            100                 105                 110

Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys
        115                 120                 125

Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys
    130                 135                 140

Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp
145                 150                 155                 160

Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met
                165                 170                 175

Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu
            180                 185                 190

Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu
        195                 200                 205

Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala
    210                 215                 220

Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp
225                 230                 235                 240

Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu
                245                 250                 255

Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu
            260                 265                 270

Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val
        275                 280                 285

Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu
    290                 295                 300

Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn
305                 310                 315                 320

Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu
                325                 330                 335
```

397/50

Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro
340 345 350

Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val
355 360 365

Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu
370 375 380

Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu
385 390 395 400

Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala
405 410 415

Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro
420 425 430

Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe
435 440 445

Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr
450 455 460

Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser
465 470 475 480

Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala
485 490 495

Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln
500 505 510

Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys
515 520 525

Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu
530 535 540

Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe
545 550 555 560

Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile
565 570 575

Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala
580 585 590

Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val
595 600 605

Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu
610 615 620

Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
625 630 635

<210> 321

<211> 638
<212> PRT
<213> Homo sapiens

<400> 321

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Met Trp Trp Arg Leu Trp Trp Leu Leu Leu Leu Leu Leu Leu Leu Trp
  1               5                  10                  15

Pro Met Val Trp Ala Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe
             20                  25                  30

Gly Arg Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys
         35                  40                  45

Val Leu Arg Arg His Asp Ala His Lys Ser Glu Val Ala His Arg Phe
     50                  55                  60

Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe
 65                  70                  75                  80

Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val
                 85                  90                  95

Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala
            100                 105                 110

Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys
        115                 120                 125

Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys
    130                 135                 140

Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp
145                 150                 155                 160

Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met
                165                 170                 175

Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu
            180                 185                 190

Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu
        195                 200                 205

Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala
    210                 215                 220

Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp
225                 230                 235                 240

Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu
                245                 250                 255

Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu
            260                 265                 270

Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val
        275                 280                 285

Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu
    290                 295                 300
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Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn
305 310 315 320

Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu
325 330 335

Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro
340 345 350

Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val
355 360 365

Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu
370 375 380

Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu
385 390 395 400

Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala
405 410 415

Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro
420 425 430

Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe
435 440 445

Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr
450 455 460

Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser
465 470 475 480

Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala
485 490 495

Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln
500 505 510

Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys
515 520 525

Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu
530 535 540

Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe
545 550 555 560

Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile
565 570 575

Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala
580 585 590

Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val
595 600 605

Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu
610 615 620

400 390

Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
625 630 635

<210> 322
<211> 666
<212> PRT
<213> Homo sapiens

<400> 322
Met Lys Val Ser Val Ala Ala Leu Ser Cys Leu Met Leu Val Thr Ala
1 5 10 15

Leu Gly Ser Gln Ala His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser
20 25 30

Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val
35 40 45

Lys Gly Arg His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr
50 55 60

Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly
65 70 75 80

Arg Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly
85 90 95

Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu
100 105 110

Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr
115 120 125

Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp
130 135 140

Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr
145 150 155 160

Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu
165 170 175

Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn
180 185 190

Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe
195 200 205

His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala
210 215 220

Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys
225 230 235 240

Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala
245 250 255

Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala
260 265 270

Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly
275 280 285

Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe
290 295 300

Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr
305 310 315 320

Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp
325 330 335

Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile
340 345 350

Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser
355 360 365

His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro
370 375 380

Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr
385 390 395 400

Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala
405 410 415

Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys
420 425 430

Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His
435 440 445

Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu
450 455 460

Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly
465 470 475 480

Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val
485 490 495

Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly
500 505 510

Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro
515 520 525

Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu
530 535 540

His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu
545 550 555 560

Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu
565 570 575

Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala
580 585 590

Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr
595 600 605

Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln
610 615 620

Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys
625 630 635 640

Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu
645 650 655

Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
660 665

<210> 323
<211> 574
<212> PRT
<213> Homo sapiens

<400> 323
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Ser Pro Lys Met Val Gln Gly Ser
20 25 30

Gly Cys Phe Gly Arg Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu
35 40 45

Gly Cys Lys Val Leu Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys
50 55 60

Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys
65 70 75 80

Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp
85 90 95

Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met
100 105 110

Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu
115 120 125

Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu
130 135 140

Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala
145 150 155 160

Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp
165 170 175

Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu
180 185 190

Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu
195 200 205

403

Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val
210 215 220

Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu
225 230 235 240

Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn
245 250 255

Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu
260 265 270

Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro
275 280 285

Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val
290 295 300

Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu
305 310 315 320

Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu
325 330 335

Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala
340 345 350

Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro
355 360 365

Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe
370 375 380

Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr
385 390 395 400

Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser
405 410 415

Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala
420 425 430

Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln
435 440 445

Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys
450 455 460

Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu
465 470 475 480

Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe
485 490 495

Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile
500 505 510

Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala
515 520 525

Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val
530 535 540

Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu
545 550 555 560

Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
565 570

<210> 324
<211> 638
<212> PRT
<213> Homo sapiens

<400> 324
Met Lys Val Ser Val Ala Ala Leu Ser Cys Leu Met Leu Val Thr Ala
1 5 10 15

Leu Gly Ser Gln Ala Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe
20 25 30

Gly Arg Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys
35 40 45

Val Leu Arg Arg His Asp Ala His Lys Ser Glu Val Ala His Arg Phe
50 55 60

Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe
65 70 75 80

Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val
85 90 95

Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala
100 105 110

Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys
115 120 125

Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys
130 135 140

Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp
145 150 155 160

Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met
165 170 175

Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu
180 185 190

Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu
195 200 205

Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala
210 215 220

Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp
225 230 235 240

Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu
245 250 255

Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu
260 265 270

Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val
275 280 285

Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu
290 295 300

Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn
305 310 315 320

Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu
325 330 335

Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro
340 345 350

Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val
355 360 365

Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu
370 375 380

Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu
385 390 395 400

Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala
405 410 415

Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro
420 425 430

Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe
435 440 445

Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr
450 455 460

Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser
465 470 475 480

Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala
485 490 495

Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln
500 505 510

Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys
515 520 525

Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu
530 535 540

Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe
545 550 555 560

406

Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile
565 570 575

Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala
580 585 590

Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val
595 600 605

Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu
610 615 620

Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
625 630 635

<210> 325
<211> 638
<212> PRT
<213> Homo sapiens

<400> 325
Met Trp Trp Arg Leu Trp Trp Leu Leu Leu Leu Leu Leu Leu Leu Trp
1 5 10 15

Pro Met Val Trp Ala Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe
20 25 30

Gly Arg Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys
35 40 45

Val Leu Arg Arg His Asp Ala His Lys Ser Glu Val Ala His Arg Phe
50 55 60

Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe
65 70 75 80

Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val
85 90 95

Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala
100 105 110

Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys
115 120 125

Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys
130 135 140

Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp
145 150 155 160

Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met
165 170 175

Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu
180 185 190

Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu
195 200 205

Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala
210 215 220

Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp
225 230 235 240

Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu
245 250 255

Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu
260 265 270

Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val
275 280 285

Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu
290 295 300

Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn
305 310 315 320

Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu
325 330 335

Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro
340 345 350

Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val
355 360 365

Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu
370 375 380

Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu
385 390 395 400

Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala
405 410 415

Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro
420 425 430

Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe
435 440 445

Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr
450 455 460

Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser
465 470 475 480

Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala
485 490 495

Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln
500 505 510

Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys
515 520 525

408.406

Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu
530 535 540

Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe
545 550 555 560

Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile
565 570 575

Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala
580 585 590

Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val
595 600 605

Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu
610 615 620

Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
625 630 635

<210> 326
<211> 640
<212> PRT
<213> Homo sapiens

<400> 326
Met Arg Pro Thr Trp Ala Trp Trp Leu Phe Leu Val Leu Leu Leu Ala
1 5 10 15

Leu Trp Ala Pro Ala Arg Gly Ser Pro Lys Met Val Gln Gly Ser Gly
20 25 30

Cys Phe Gly Arg Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly
35 40 45

Cys Lys Val Leu Arg Arg His Asp Ala His Lys Ser Glu Val Ala His
50 55 60

Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile
65 70 75 80

Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys
85 90 95

Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu
100 105 110

Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys
115 120 125

Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp
130 135 140

Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His
145 150 155 160

Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp
165 170 175

Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys
180 185 190

Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu
195 200 205

Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys
210 215 220

Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu
225 230 235 240

Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala
245 250 255

Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala
260 265 270

Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys
275 280 285

Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp
290 295 300

Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys
305 310 315 320

Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys
325 330 335

Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu
340 345 350

Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys
355 360 365

Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met
370 375 380

Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu
385 390 395 400

Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys
405 410 415

Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe
420 425 430

Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu
435 440 445

Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val
450 455 460

Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu
465 470 475 480

Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro
485 490 495

410

```
Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu
            500                 505                 510

Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val
        515                 520                 525

Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser
    530                 535                 540

Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu
545                 550                 555                 560

Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg
                565                 570                 575

Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro
            580                 585                 590

Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala
        595                 600                 605

Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala
    610                 615                 620

Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
625                 630                 635                 640


<210> 327
<211> 640
<212> PRT
<213> Homo sapiens

<400> 327
Met Arg Pro Thr Trp Ala Trp Trp Leu Phe Leu Val Leu Leu Leu Ala
  1               5                  10                  15

Leu Trp Ala Pro Ala Arg Gly Ser Pro Lys Met Val Gln Gly Ser Gly
             20                  25                  30

Cys Phe Gly Arg Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly
         35                  40                  45

Cys Lys Val Leu Arg Arg His Asp Ala His Lys Ser Glu Val Ala His
     50                  55                  60

Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile
 65                  70                  75                  80

Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys
                 85                  90                  95

Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu
            100                 105                 110

Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys
        115                 120                 125

Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp
    130                 135                 140
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Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His
145 150 155 160

Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp
165 170 175

Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys
180 185 190

Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu
195 200 205

Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys
210 215 220

Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu
225 230 235 240

Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala
245 250 255

Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala
260 265 270

Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys
275 280 285

Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp
290 295 300

Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys
305 310 315 320

Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys
325 330 335

Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu
340 345 350

Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys
355 360 365

Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met
370 375 380

Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu
385 390 395 400

Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys
405 410 415

Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe
420 425 430

Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu
435 440 445

Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val
450 455 460

Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu

465 470 475 480

Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro
485 490 495

Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu
500 505 510

Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val
515 520 525

Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser
530 535 540

Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu
545 550 555 560

Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg
565 570 575

Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro
580 585 590

Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala
595 600 605

Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala
610 615 620

Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
625 630 635 640

<210> 328
<211> 638
<212> PRT
<213> Homo sapiens

<400> 328
Met Lys Val Ser Val Ala Ala Leu Ser Cys Leu Met Leu Val Thr Ala
1 5 10 15

Leu Gly Ser Gln Ala Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe
20 25 30

Gly Gly Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys
35 40 45

Val Leu Arg Arg His Asp Ala His Lys Ser Glu Val Ala His Arg Phe
50 55 60

Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe
65 70 75 80

Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val
85 90 95

Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala
100 105 110

Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys

```
        115                 120                 125

Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys
    130                 135                 140

Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp
145                 150                 155                 160

Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met
                165                 170                 175

Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu
            180                 185                 190

Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu
        195                 200                 205

Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala
    210                 215                 220

Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp
225                 230                 235                 240

Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu
                245                 250                 255

Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu
            260                 265                 270

Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val
        275                 280                 285

Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu
    290                 295                 300

Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn
305                 310                 315                 320

Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu
                325                 330                 335

Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro
            340                 345                 350

Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val
        355                 360                 365

Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu
    370                 375                 380

Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu
385                 390                 395                 400

Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala
                405                 410                 415

Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro
            420                 425                 430

Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe
        435                 440                 445
```

Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr
450 455 460

Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser
465 470 475 480

Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala
485 490 495

Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln
500 505 510

Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys
515 520 525

Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu
530 535 540

Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe
545 550 555 560

Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile
565 570 575

Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala
580 585 590

Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val
595 600 605

Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu
610 615 620

Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
625 630 635

<210> 329
<211> 571
<212> PRT
<213> Homo sapiens

<400> 329
Met Trp Trp Arg Leu Trp Trp Leu Leu Leu Leu Leu Leu Leu Leu Trp
1 5 10 15

Pro Met Val Trp Ala Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe
20 25 30

Gly Arg Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys
35 40 45

Val Leu Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala
50 55 60

Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln
65 70 75 80

Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro
85 90 95

Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala
100 105 110

Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile
115 120 125

Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala
130 135 140

Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys
145 150 155 160

Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys
165 170 175

Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe
180 185 190

Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg
195 200 205

Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu
210 215 220

Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala
225 230 235 240

Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser
245 250 255

Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys
260 265 270

Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu
275 280 285

Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn
290 295 300

Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr
305 310 315 320

Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala
325 330 335

Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro
340 345 350

His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu
355 360 365

Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu
370 375 380

Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys
385 390 395 400

Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu
405 410 415

```
Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met
            420                 425                 430

Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val
        435                 440                 445

Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr
    450                 455                 460

Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp
465                 470                 475                 480

Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His
                485                 490                 495

Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln
            500                 505                 510

Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu
        515                 520                 525

Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys
    530                 535                 540

Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys
545                 550                 555                 560

Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
                565                 570
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<210> 330
<211> 638
<212> PRT
<213> Homo sapiens

<400> 330

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Met Trp Trp Arg Leu Trp Trp Leu Leu Leu Leu Leu Leu Leu Trp
  1               5                  10                  15

Pro Met Val Trp Ala Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe
            20                  25                  30

Gly Gly Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys
        35                  40                  45

Val Leu Arg Arg His Asp Ala His Lys Ser Glu Val Ala His Arg Phe
    50                  55                  60

Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe
65                  70                  75                  80

Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val
                85                  90                  95

Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala
            100                 105                 110

Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys
        115                 120                 125
```

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Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys
    130                 135                 140

Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp
145                 150                 155                 160

Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met
                165                 170                 175

Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu
            180                 185                 190

Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu
        195                 200                 205

Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala
    210                 215                 220

Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp
225                 230                 235                 240

Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu
                245                 250                 255

Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu
            260                 265                 270

Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val
        275                 280                 285

Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu
    290                 295                 300

Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn
305                 310                 315                 320

Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu
                325                 330                 335

Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro
            340                 345                 350

Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val
        355                 360                 365

Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu
    370                 375                 380

Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu
385                 390                 395                 400

Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala
                405                 410                 415

Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro
            420                 425                 430

Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe
        435                 440                 445
```

Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr
450 455 460

Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser
465 470 475 480

Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala
485 490 495

Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln
500 505 510

Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys
515 520 525

Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu
530 535 540

Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe
545 550 555 560

Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile
565 570 575

Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala
580 585 590

Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val
595 600 605

Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu
610 615 620

Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
625 630 635

<210> 331
<211> 638
<212> PRT
<213> Homo sapiens

<400> 331
Met Trp Trp Arg Leu Trp Trp Leu Leu Leu Leu Leu Leu Leu Leu Trp
1 5 10 15

Pro Met Val Trp Ala Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe
20 25 30

Gly Arg Gly Met Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys
35 40 45

Val Leu Arg Arg His Asp Ala His Lys Ser Glu Val Ala His Arg Phe
50 55 60

Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe
65 70 75 80

Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val
85 90 95

Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala
100 105 110

Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys
115 120 125

Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys
130 135 140

Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp
145 150 155 160

Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met
165 170 175

Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu
180 185 190

Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu
195 200 205

Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala
210 215 220

Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp
225 230 235 240

Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu
245 250 255

Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu
260 265 270

Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val
275 280 285

Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu
290 295 300

Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn
305 310 315 320

Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu
325 330 335

Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro
340 345 350

Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val
355 360 365

Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu
370 375 380

Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu
385 390 395 400

Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala
405 410 415

Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro
420 425 430

Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe
435 440 445

Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr
450 455 460

Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser
465 470 475 480

Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala
485 490 495

Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln
500 505 510

Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys
515 520 525

Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu
530 535 540

Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe
545 550 555 560

Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile
565 570 575

Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala
580 585 590

Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val
595 600 605

Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu
610 615 620

Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
625 630 635

<210> 332
<211> 641
<212> PRT
<213> Homo sapiens

<400> 332
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu

385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met
610 615 620

Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg
625 630 635 640

His

<210> 333
<211> 642
<212> PRT
<213> Homo sapiens

<400> 333
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Ser Pro Lys Met Val Gln Gly Ser



20 25 30

Gly Cys Phe Gly Arg Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu
35 40 45

Gly Cys Lys Val Leu Arg Gly Gly Gly Asp Ala His Lys Ser Glu Val
50 55 60

Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val
65 70 75 80

Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His
85 90 95

Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala
100 105 110

Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly
115 120 125

Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met
130 135 140

Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu
145 150 155 160

Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu
165 170 175

Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu
180 185 190

Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala
195 200 205

Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu
210 215 220

Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp
225 230 235 240

Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys
245 250 255

Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala
260 265 270

Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val
275 280 285

Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His
290 295 300

Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr
305 310 315 320

Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys
325 330 335

Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn
340 345 350

Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu
355 360 365

Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu
370 375 380

Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val
385 390 395 400

Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys
405 410 415

Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp
420 425 430

Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn
435 440 445

Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu
450 455 460

Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu
465 470 475 480

Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys
485 490 495

His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val
500 505 510

Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp
515 520 525

Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys
530 535 540

Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn
545 550 555 560

Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys
565 570 575

Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His
580 585 590

Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe
595 600 605

Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys
610 615 620

Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu
625 630 635 640

Gly Leu

<210> 334

<211> 639
<212> PRT
<213> Homo sapiens

<400> 334

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Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
  1               5                  10                  15

Tyr Ser Arg Ser Leu Asp Lys Arg Asn Pro Met Tyr Asn Ala Val Ser
             20                  25                  30

Asn Ala Asp Leu Met Asp Phe Lys Asn Leu Leu Asp His Leu Glu Glu
         35                  40                  45

Lys Met Pro Leu Glu Asp Asp Ala His Lys Ser Glu Val Ala His Arg
     50                  55                  60

Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala
 65                  70                  75                  80

Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu
                 85                  90                  95

Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser
            100                 105                 110

Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu
        115                 120                 125

Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys
    130                 135                 140

Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys
145                 150                 155                 160

Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val
                165                 170                 175

Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr
            180                 185                 190

Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu
        195                 200                 205

Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln
    210                 215                 220

Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg
225                 230                 235                 240

Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser
                245                 250                 255

Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg
            260                 265                 270

Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu
        275                 280                 285

Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu
    290                 295                 300
```

Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu
305 310 315 320

Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro
325 330 335

Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met
340 345 350

Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp
355 360 365

Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe
370 375 380

Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu
385 390 395 400

Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala
405 410 415

Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys
420 425 430

Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu
435 440 445

Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg
450 455 460

Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val
465 470 475 480

Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu
485 490 495

Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn
500 505 510

Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr
515 520 525

Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala
530 535 540

Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr
545 550 555 560

Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln
565 570 575

Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys
580 585 590

Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe
595 600 605

Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu
610 615 620

Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
625 630 635

<210> 335
<211> 639
<212> PRT
<213> Homo sapiens

<400> 335
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Asn Pro Met Tyr Asn Ala Val Ser Asn Ala Asp Leu Met Asp Phe
610 615 620

Lys Asn Leu Leu Asp His Leu Glu Glu Lys Met Pro Leu Glu Asp
625 630 635

<210> 336
<211> 646
<212> PRT
<213> Homo sapiens

<400> 336
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Glu Val Val Pro Pro Gln Val Leu
20 25 30

Ser Glu Pro Asn Glu Glu Ala Gly Ala Ala Leu Ser Pro Leu Pro Glu
35 40 45

Val Pro Pro Trp Thr Gly Glu Val Ser Pro Ala Gln Arg Asp Ala His
50 55 60

Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe
65 70 75 80

Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro
85 90 95

Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys
100 105 110

Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His
115 120 125

Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr
130 135 140

Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn
145 150 155 160

Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu
165 170 175

Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu
180 185 190

Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro
195 200 205

Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala
210 215 220

Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu
225 230 235 240

Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys
245 250 255

Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe
260 265 270

Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu
275 280 285

Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr
290 295 300

Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp
305 310 315 320

Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu
325 330 335

Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala
340 345 350

Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala
355 360 365

Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys
370 375 380

Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro
385 390 395 400

Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr
405 410 415

Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala
420 425 430

Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu
435 440 445

Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe
450 455 460

Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser
465 470 475 480

Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser
485 490 495

Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp
500 505 510

Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr
515 520 525

Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn
530 535 540

Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro
545 550 555 560

Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr
565 570 575

Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu
580 585 590

Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val
595 600 605

Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp
610 615 620

Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser
625 630 635 640

Gln Ala Ala Leu Gly Leu
645

<210> 337
<211> 646
<212> PRT
<213> Homo sapiens

<400> 337
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Glu Val Val Pro Pro Gln Val Leu Ser Glu Pro Asn Glu Glu Ala
610 615 620

Gly Ala Ala Leu Ser Pro Leu Pro Glu Val Pro Pro Trp Thr Gly Glu
625 630 635 640

Val Ser Pro Ala Gln Arg
645

<210> 338
<211> 629
<212> PRT
<213> Homo sapiens

<400> 338
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Ser Ser Asp Arg Ser Ala Leu Leu
20 25 30

Lys Ser Lys Leu Arg Ala Leu Leu Thr Ala Pro Arg Asp Ala His Lys
35 40 45

Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys
50 55 60

Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe
65 70 75 80

Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr
85 90 95

Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr
100 105 110

Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr
115 120 125

Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu
130 135 140

Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val
145 150 155 160

Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu
165 170 175

Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr
180 185 190

Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala
195 200 205

Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro
210 215 220

Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln
225 230 235 240

Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys
245 250 255

Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe
260 265 270

Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu
275 280 285

Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu
290 295 300

Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys
305 310 315 320

Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu
325 330 335

Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp
340 345 350

Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp
355 360 365

Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp
370 375 380

Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr
385 390 395 400

Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys
405 410 415

Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile
420 425 430

Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln
435 440 445

Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr
450 455 460

435

Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys
465 470 475 480

Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr
485 490 495

Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro
500 505 510

Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg
515 520 525

Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys
530 535 540

Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu
545 550 555 560

Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu
565 570 575

Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met
580 585 590

Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys
595 600 605

Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln
610 615 620

Ala Ala Leu Gly Leu
625

<210> 339
<211> 629
<212> PRT
<213> Homo sapiens

<400> 339
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110



Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Ser Ser Asp Arg Ser Ala Leu Leu Lys Ser Lys Leu Arg Ala Leu
610 615 620

Leu Thr Ala Pro Arg
625

<210> 340
<211> 631
<212> PRT
<213> Homo sapiens

<400> 340
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Gly Leu Ser Lys Gly Cys Phe Gly
20 25 30

Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys Asp Ala
35 40 45

His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn
50 55 60

Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys
65 70 75 80

Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala
85 90 95

Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu
100 105 110

His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu
115 120 125

Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg
130 135 140

Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg
145 150 155 160

Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn
165 170 175

Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His
180 185 190

Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys
195 200 205

Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu
210 215 220

Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala
225 230 235 240

Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala
245 250 255

Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala
260 265 270

Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His
275 280 285

Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala
290 295 300

Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys
305 310 315 320

Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile
325 330 335

Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala
340 345 350

Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala
355 360 365

Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His
370 375 380

Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu
385 390 395 400

Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr
405 410 415

Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn
420 425 430

Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys
435 440 445

Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val
450 455 460

Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly
465 470 475 480

Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu
485 490 495

Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys
500 505 510

Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val
515 520 525

Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val
530 535 540

Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys
545 550 555 560

Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val
565 570 575

Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala
580 585 590

Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp
595 600 605

Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala
610 615 620

Ser Gln Ala Ala Leu Gly Leu
625 630

<210> 341
<211> 631
<212> PRT
<213> Homo sapiens

<400> 341
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

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Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
     50                  55                  60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
 65                  70                  75                  80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
                 85                  90                  95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
            100                 105                 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
        115                 120                 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
    130                 135                 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145                 150                 155                 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
                165                 170                 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
            180                 185                 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
        195                 200                 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
    210                 215                 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225                 230                 235                 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
                245                 250                 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
            260                 265                 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
        275                 280                 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
    290                 295                 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305                 310                 315                 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
                325                 330                 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
            340                 345                 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
        355                 360                 365
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Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Gly Leu Ser Lys Gly Cys Phe Gly Leu Lys Leu Asp Arg Ile Gly
610 615 620

Ser Met Ser Gly Leu Gly Cys
625 630

<210> 342
<211> 647
<212> PRT
<213> Homo sapiens

<400> 342
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Glu Val Lys Tyr Asp Pro Cys Phe
20 25 30

Gly His Lys Ile Asp Arg Ile Asn His Val Ser Asn Leu Gly Cys Pro
35 40 45

Ser Leu Arg Asp Pro Arg Pro Asn Ala Pro Ser Thr Ser Ala Asp Ala
50 55 60

His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn
65 70 75 80

Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys
85 90 95

Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala
100 105 110

Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu
115 120 125

His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu
130 135 140

Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg
145 150 155 160

Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg
165 170 175

Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn
180 185 190

Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His
195 200 205

Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys
210 215 220

Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu
225 230 235 240

Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala
245 250 255

Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala
260 265 270

Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala
275 280 285

Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His
290 295 300

Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala
305 310 315 320

Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys
325 330 335

Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile
340 345 350

Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala
355 360 365

Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala
370 375 380

Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His
385 390 395 400

Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu
405 410 415

Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr
420 425 430

Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn
435 440 445

Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys
450 455 460

Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val
465 470 475 480

Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly
485 490 495

Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu
500 505 510

Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys
515 520 525

Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val
530 535 540

Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val
545 550 555 560

Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys
565 570 575

Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val
580 585 590

Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala
595 600 605

Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp
610 615 620

Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala
625 630 635 640

Ser Gln Ala Ala Leu Gly Leu
645

<210> 343
<211> 647
<212> PRT
<213> Homo sapiens

<400> 343

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Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
  1               5                  10                  15

Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
             20                  25                  30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
         35                  40                  45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
     50                  55                  60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
 65                  70                  75                  80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
                 85                  90                  95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
            100                 105                 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
        115                 120                 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
    130                 135                 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145                 150                 155                 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
                165                 170                 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
            180                 185                 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
        195                 200                 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
    210                 215                 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225                 230                 235                 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
                245                 250                 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
            260                 265                 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
        275                 280                 285
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Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Glu Val Lys Tyr Asp Pro Cys Phe Gly His Lys Ile Asp Arg Ile

610 615 620

Asn His Val Ser Asn Leu Gly Cys Pro Ser Leu Arg Asp Pro Arg Pro
625 630 635 640

Asn Ala Pro Ser Thr Ser Ala
645

<210> 344
<211> 642
<212> PRT
<213> Homo sapiens

<400> 344
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Gly Ser Leu Asp Lys Arg Ser Pro Lys Met Val Gln Gly Ser
20 25 30

Gly Cys Phe Gly Arg Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu
35 40 45

Gly Cys Lys Val Leu Arg Gly Gly Gly Asp Ala His Lys Ser Glu Val
50 55 60

Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val
65 70 75 80

Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His
85 90 95

Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala
100 105 110

Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly
115 120 125

Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met
130 135 140

Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu
145 150 155 160

Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu
165 170 175

Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu
180 185 190

Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala
195 200 205

Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu
210 215 220

Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp
225 230 235 240

Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys
245 250 255

Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala
260 265 270

Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val
275 280 285

Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His
290 295 300

Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr
305 310 315 320

Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys
325 330 335

Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn
340 345 350

Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu
355 360 365

Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu
370 375 380

Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val
385 390 395 400

Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys
405 410 415

Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp
420 425 430

Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn
435 440 445

Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu
450 455 460

Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu
465 470 475 480

Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys
485 490 495

His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val
500 505 510

Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp
515 520 525

Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys
530 535 540

Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn
545 550 555 560

Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys

565 570 575

Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His
580 585 590

Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe
595 600 605

Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys
610 615 620

Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu
625 630 635 640

Gly Leu

<210> 345
<211> 638
<212> PRT
<213> Homo sapiens

<400> 345
Met Trp Trp Arg Leu Trp Trp Leu Leu Leu Leu Leu Leu Leu Leu Trp
1 5 10 15

Pro Met Val Trp Ala Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe
20 25 30

Gly Arg Gly Met Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys
35 40 45

Val Leu Arg Arg His Asp Ala His Lys Ser Glu Val Ala His Arg Phe
50 55 60

Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe
65 70 75 80

Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val
85 90 95

Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala
100 105 110

Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys
115 120 125

Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys
130 135 140

Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp
145 150 155 160

Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met
165 170 175

Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu
180 185 190

Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu
195 200 205

Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala
210 215 220

Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp
225 230 235 240

Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu
245 250 255

Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu
260 265 270

Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val
275 280 285

Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu
290 295 300

Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn
305 310 315 320

Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu
325 330 335

Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro
340 345 350

Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val
355 360 365

Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu
370 375 380

Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu
385 390 395 400

Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala
405 410 415

Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro
420 425 430

Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe
435 440 445

Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr
450 455 460

Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser
465 470 475 480

Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala
485 490 495

Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln
500 505 510

Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys
515 520 525

448
456

Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu
530 535 540

Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe
545 550 555 560

Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile
565 570 575

Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala
580 585 590

Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val
595 600 605

Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu
610 615 620

Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
625 630 635

<210> 346
<211> 641
<212> PRT
<213> Homo sapiens

<400> 346
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Ser Pro Lys Met Val Gln Gly Ser
20 25 30

Gly Cys Phe Gly Arg Gly Met Asp Arg Ile Ser Ser Ser Ser Gly Leu
35 40 45

Gly Cys Lys Val Leu Arg Arg His Asp Ala His Lys Ser Glu Val Ala
50 55 60

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
65 70 75 80

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
85 90 95

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
100 105 110

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
115 120 125

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
130 135 140

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
145 150 155 160

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
165 170 175

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
180 185 190

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
195 200 205

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
210 215 220

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
225 230 235 240

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
245 250 255

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
260 265 270

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
275 280 285

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
290 295 300

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
305 310 315 320

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
325 330 335

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
340 345 350

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
355 360 365

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
370 375 380

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
385 390 395 400

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
405 410 415

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
420 425 430

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
435 440 445

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
450 455 460

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
465 470 475 480

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
485 490 495

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
500 505 510

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
515 520 525

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
530 535 540

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
545 550 555 560

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
565 570 575

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
580 585 590

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
595 600 605

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
610 615 620

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
625 630 635 640

Leu

<210> 347
<211> 641
<212> PRT
<213> Homo sapiens

<400> 347
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Gly Ser Leu Asp Lys Arg Ser Pro Lys Met Val Gln Gly Ser
20 25 30

Gly Cys Phe Gly Arg Gly Met Asp Arg Ile Ser Ser Ser Ser Gly Leu
35 40 45

Gly Cys Lys Val Leu Arg Arg His Asp Ala His Lys Ser Glu Val Ala
50 55 60

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
65 70 75 80

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
85 90 95

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
100 105 110

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
115 120 125

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala

130 135 140

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
145 150 155 160

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
165 170 175

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
180 185 190

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
195 200 205

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
210 215 220

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
225 230 235 240

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
245 250 255

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
260 265 270

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
275 280 285

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
290 295 300

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
305 310 315 320

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
325 330 335

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
340 345 350

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
355 360 365

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
370 375 380

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
385 390 395 400

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
405 410 415

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
420 425 430

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
435 440 445

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
450 455 460

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
465 470 475 480

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
485 490 495

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
500 505 510

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
515 520 525

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
530 535 540

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
545 550 555 560

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
565 570 575

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
580 585 590

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
595 600 605

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
610 615 620

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
625 630 635 640

Leu

<210> 348
<211> 641
<212> PRT
<213> Homo sapiens

<400> 348
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp

85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Gly Met
610 615 620

Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg
625 630 635 640

His

<210> 349
<211> 909
<212> PRT
<213> Homo sapiens

<400> 349
Met Trp Trp Arg Leu Trp Trp Leu Leu Leu Leu Leu Leu Leu Leu Trp
1 5 10 15

Pro Met Val Trp Ala Met Ala His Tyr His Asp Asp Tyr Gly Lys Asn
20 25 30

Asp Glu Val Glu Phe Val Arg Thr Gly Tyr Gly Lys Asp Met Val Lys
35 40 45

Val Leu His Ile Gln Arg Asp Gly Lys Tyr His Ser Ile Lys Glu Val
50 55 60

Ala Thr Ser Val Gln Leu Thr Leu Arg Ser Lys Lys Asp Tyr Leu His
65 70 75 80

Gly Asp Asn Ser Asp Ile Ile Pro Thr Asp Thr Ile Lys Asn Thr Val
85 90 95

His Val Leu Ala Lys Phe Lys Gly Ile Lys Ser Ile Glu Thr Phe Ala
100 105 110

Met Asn Ile Cys Glu His Phe Leu Ser Ser Phe Ser His Val Thr Arg
115 120 125

Ala His Val Tyr Val Glu Glu Val Pro Trp Lys Arg Phe Glu Lys Asn
130 135 140

Gly Val Lys His Val His Ala Phe Ile His Thr Pro Thr Gly Thr His
145 150 155 160

Phe Cys Asp Val Glu Gln Val Arg Asn Gly Pro Pro Ile Ile His Ser
165 170 175

Gly Ile Lys Asp Leu Lys Val Leu Lys Thr Thr Gln Ser Gly Phe Glu
180 185 190

Gly Phe Ile Lys Asp Gln Phe Thr Thr Leu Pro Glu Val Lys Asp Arg
195 200 205

Cys Phe Ala Thr Gln Val Tyr Cys Lys Trp Arg Tyr Gln Asn Arg Asp
210 215 220

Val Asp Phe Glu Ala Thr Trp Gly Ala Val Arg Asp Ile Val Leu Lys
225 230 235 240

Lys Phe Ala Gly Pro Tyr Asp Arg Gly Glu Tyr Ser Pro Ser Val Gln
245 250 255

Lys Thr Leu Tyr Asp Ile Gln Val Leu Thr Leu Ser Gln Leu Pro Glu
260 265 270

Ile Glu Asp Met Glu Ile Ser Leu Pro Asn Ile His Tyr Phe Asn Ile
275 280 285

Asp Met Ser Lys Met Gly Leu Ile Asn Lys Glu Glu Val Leu Leu Pro
290 295 300

Leu Asp Asn Pro Tyr Gly Lys Ile Thr Gly Thr Val Arg Arg Lys Leu
305 310 315 320

Pro Ser Arg Leu Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys
325 330 335

Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala
340 345 350

Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn
355 360 365

Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu
370 375 380

Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr
385 390 395 400

Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala
405 410 415

Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp
420 425 430

Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys
435 440 445

Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr
450 455 460

Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe
465 470 475 480

Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala
485 490 495

Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu
500 505 510

Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln
515 520 525

Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser
530 535 540

Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr
545 550 555 560

Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu
565 570 575

Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln
580 585 590

Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu
595 600 605

Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala
610 615 620

Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys
625 630 635 640

Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr
645 650 655

Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg
660 665 670

Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala
675 680 685

Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu

690 695 700

Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu
705 710 715 720

Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr
725 730 735

Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg
740 745 750

Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys
755 760 765

Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu
770 775 780

Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys
785 790 795 800

Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu
805 810 815

Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr
820 825 830

Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys
835 840 845

Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr
850 855 860

Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu
865 870 875 880

Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly
885 890 895

Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
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<400> 350
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Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp

65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Met Ala His Tyr His Asp Asp Tyr Gly Lys Asn Asp Glu Val Glu
610 615 620

Phe Val Arg Thr Gly Tyr Gly Lys Asp Met Val Lys Val Leu His Ile
625 630 635 640

Gln Arg Asp Gly Lys Tyr His Ser Ile Lys Glu Val Ala Thr Ser Val
645 650 655

Gln Leu Thr Leu Arg Ser Lys Lys Asp Tyr Leu His Gly Asp Asn Ser
660 665 670

Asp Ile Ile Pro Thr Asp Thr Ile Lys Asn Thr Val His Val Leu Ala
675 680 685

Lys Phe Lys Gly Ile Lys Ser Ile Glu Thr Phe Ala Met Asn Ile Cys
690 695 700

Glu His Phe Leu Ser Ser Phe Ser His Val Thr Arg Ala His Val Tyr
705 710 715 720

Val Glu Glu Val Pro Trp Lys Arg Phe Glu Lys Asn Gly Val Lys His
725 730 735

Val His Ala Phe Ile His Thr Pro Thr Gly Thr His Phe Cys Asp Val
740 745 750

Glu Gln Val Arg Asn Gly Pro Pro Ile Ile His Ser Gly Ile Lys Asp
755 760 765

Leu Lys Val Leu Lys Thr Thr Gln Ser Gly Phe Glu Gly Phe Ile Lys
770 775 780

Asp Gln Phe Thr Thr Leu Pro Glu Val Lys Asp Arg Cys Phe Ala Thr
785 790 795 800

Gln Val Tyr Cys Lys Trp Arg Tyr Gln Asn Arg Asp Val Asp Phe Glu
805 810 815

Ala Thr Trp Gly Ala Val Arg Asp Ile Val Leu Lys Lys Phe Ala Gly
820 825 830

Pro Tyr Asp Arg Gly Glu Tyr Ser Pro Ser Val Gln Lys Thr Leu Tyr
835 840 845

Asp Ile Gln Val Leu Thr Leu Ser Gln Leu Pro Glu Ile Glu Asp Met
850 855 860

Glu Ile Ser Leu Pro Asn Ile His Tyr Phe Asn Ile Asp Met Ser Lys
865 870 875 880

Met Gly Leu Ile Asn Lys Glu Glu Val Leu Leu Pro Leu Asp Asn Pro
885 890 895

Tyr Gly Lys Ile Thr Gly Thr Val Arg Arg Lys Leu Pro Ser Arg Leu
900 905 910

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Tyr Ser Arg Ser Leu Asp Lys Arg Met Ser Val Phe Asp Ser Lys Phe
20 25 30

Lys Gly Ile His Val Tyr Ser Glu Ile Gly Glu Leu Glu Ser Val Leu
35 40 45

Val His Glu Pro Gly Arg Glu Ile Asp Tyr Ile Thr Pro Ala Arg Leu
50 55 60

Asp Glu Leu Leu Phe Ser Ala Ile Leu Glu Ser His Asp Ala Arg Lys
65 70 75 80

Glu His Lys Gln Phe Val Ala Glu Leu Lys Ala Asn Asp Ile Asn Val

85 90 95

Val Glu Leu Ile Asp Leu Val Ala Glu Thr Tyr Asp Leu Ala Ser Gln
100 105 110

Glu Ala Lys Asp Lys Leu Ile Glu Glu Phe Leu Glu Asp Ser Glu Pro
115 120 125

Val Leu Ser Glu Glu His Lys Val Val Val Arg Asn Phe Leu Lys Ala
130 135 140

Lys Lys Thr Ser Arg Lys Leu Val Glu Ile Met Met Ala Gly Ile Thr
145 150 155 160

Lys Tyr Asp Leu Gly Ile Glu Ala Asp His Glu Leu Ile Val Asp Pro
165 170 175

Met Pro Asn Leu Tyr Phe Thr Arg Asp Pro Phe Ala Ser Val Gly Asn
180 185 190

Gly Val Thr Ile His Tyr Met Arg Tyr Lys Val Arg Gln Arg Glu Thr
195 200 205

Leu Phe Ser Arg Phe Val Phe Ser Asn His Pro Lys Leu Ile Asn Thr
210 215 220

Pro Trp Tyr Tyr Asp Pro Ser Leu Lys Leu Ser Ile Glu Gly Gly Asp
225 230 235 240

Val Phe Ile Tyr Asn Asn Asp Thr Leu Val Val Gly Val Ser Glu Arg
245 250 255

Thr Asp Leu Gln Thr Val Thr Leu Leu Ala Lys Asn Ile Val Ala Asn
260 265 270

Lys Glu Cys Glu Phe Lys Arg Ile Val Ala Ile Asn Val Pro Lys Trp
275 280 285

Thr Asn Leu Met His Leu Asp Thr Trp Leu Thr Met Leu Asp Lys Asp
290 295 300

Lys Phe Leu Tyr Ser Pro Ile Ala Asn Asp Val Phe Lys Phe Trp Asp
305 310 315 320

Tyr Asp Leu Val Asn Gly Gly Ala Glu Pro Gln Pro Val Glu Asn Gly
325 330 335

Leu Pro Leu Glu Gly Leu Leu Gln Ser Ile Ile Asn Lys Lys Pro Val
340 345 350

Leu Ile Pro Ile Ala Gly Glu Gly Ala Ser Gln Met Glu Ile Glu Arg
355 360 365

Glu Thr His Phe Asp Gly Thr Asn Tyr Leu Ala Ile Arg Pro Gly Val
370 375 380

Val Ile Gly Tyr Ser Arg Asn Glu Lys Thr Asn Ala Ala Leu Glu Ala
385 390 395 400

Ala Gly Ile Lys Val Leu Pro Phe His Gly Asn Gln Leu Ser Leu Gly
405 410 415

Met Gly Asn Ala Arg Cys Met Ser Met Pro Leu Ser Arg Lys Asp Val
420 425 430

Lys Trp Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu
435 440 445

Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr
450 455 460

Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val
465 470 475 480

Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys
485 490 495

Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala
500 505 510

Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln
515 520 525

Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro
530 535 540

Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala
545 550 555 560

Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile
565 570 575

Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala
580 585 590

Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys
595 600 605

Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys
610 615 620

Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe
625 630 635 640

Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg
645 650 655

Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu
660 665 670

Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala
675 680 685

Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser
690 695 700

Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys
705 710 715 720

Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu
725 730 735

Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn
740 745 750

Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr
755 760 765

Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala
770 775 780

Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro
785 790 795 800

His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu
805 810 815

Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu
820 825 830

Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys
835 840 845

Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu
850 855 860

Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met
865 870 875 880

Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val
885 890 895

Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr
900 905 910

Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp
915 920 925

Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His
930 935 940

Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln
945 950 955 960

Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu
965 970 975

Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys
980 985 990

Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys
995 1000 1005

Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
1010 1015

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<213> Homo sapiens

<400> 352

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Pro Met Val Trp Ala Met Ser Val Phe Asp Ser Lys Phe Lys Gly Ile
             20                  25                  30

His Val Tyr Ser Glu Ile Gly Glu Leu Glu Ser Val Leu Val His Glu
         35                  40                  45

Pro Gly Arg Glu Ile Asp Tyr Ile Thr Pro Ala Arg Leu Asp Glu Leu
     50                  55                  60

Leu Phe Ser Ala Ile Leu Glu Ser His Asp Ala Arg Lys Glu His Lys
 65                  70                  75                  80

Gln Phe Val Ala Glu Leu Lys Ala Asn Asp Ile Asn Val Val Glu Leu
                 85                  90                  95

Ile Asp Leu Val Ala Glu Thr Tyr Asp Leu Ala Ser Gln Glu Ala Lys
            100                 105                 110

Asp Lys Leu Ile Glu Glu Phe Leu Glu Asp Ser Glu Pro Val Leu Ser
        115                 120                 125

Glu Glu His Lys Val Val Val Arg Asn Phe Leu Lys Ala Lys Lys Thr
    130                 135                 140

Ser Arg Lys Leu Val Glu Ile Met Met Ala Gly Ile Thr Lys Tyr Asp
145                 150                 155                 160

Leu Gly Ile Glu Ala Asp His Glu Leu Ile Val Asp Pro Met Pro Asn
                165                 170                 175

Leu Tyr Phe Thr Arg Asp Pro Phe Ala Ser Val Gly Asn Gly Val Thr
            180                 185                 190

Ile His Tyr Met Arg Tyr Lys Val Arg Gln Arg Glu Thr Leu Phe Ser
        195                 200                 205

Arg Phe Val Phe Ser Asn His Pro Lys Leu Ile Asn Thr Pro Trp Tyr
    210                 215                 220

Tyr Asp Pro Ser Leu Lys Leu Ser Ile Glu Gly Gly Asp Val Phe Ile
225                 230                 235                 240

Tyr Asn Asn Asp Thr Leu Val Val Gly Val Ser Glu Arg Thr Asp Leu
                245                 250                 255

Gln Thr Val Thr Leu Leu Ala Lys Asn Ile Val Ala Asn Lys Glu Cys
            260                 265                 270

Glu Phe Lys Arg Ile Val Ala Ile Asn Val Pro Lys Trp Thr Asn Leu
        275                 280                 285

Met His Leu Asp Thr Trp Leu Thr Met Leu Asp Lys Asp Lys Phe Leu
    290                 295                 300

Tyr Ser Pro Ile Ala Asn Asp Val Phe Lys Phe Trp Asp Tyr Asp Leu
305                 310                 315                 320
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Val Asn Gly Gly Ala Glu Pro Gln Pro Val Glu Asn Gly Leu Pro Leu
325 330 335

Glu Gly Leu Leu Gln Ser Ile Ile Asn Lys Lys Pro Val Leu Ile Pro
340 345 350

Ile Ala Gly Glu Gly Ala Ser Gln Met Glu Ile Glu Arg Glu Thr His
355 360 365

Phe Asp Gly Thr Asn Tyr Leu Ala Ile Arg Pro Gly Val Val Ile Gly
370 375 380

Tyr Ser Arg Asn Glu Lys Thr Asn Ala Ala Leu Glu Ala Ala Gly Ile
385 390 395 400

Lys Val Leu Pro Phe His Gly Asn Gln Leu Ser Leu Gly Met Gly Asn
405 410 415

Ala Arg Cys Met Ser Met Pro Leu Ser Arg Lys Asp Val Lys Trp Asp
420 425 430

Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu
435 440 445

Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln
450 455 460

Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe
465 470 475 480

Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser
485 490 495

Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg
500 505 510

Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu
515 520 525

Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro
530 535 540

Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp
545 550 555 560

Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg
565 570 575

His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr
580 585 590

Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys
595 600 605

Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser
610 615 620

Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg
625 630 635 640

Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys

645 650 655

Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val
660 665 670

His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg
675 680 685

Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser
690 695 700

Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys
705 710 715 720

Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu
725 730 735

Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu
740 745 750

Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg
755 760 765

His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr
770 775 780

Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys
785 790 795 800

Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln
805 810 815

Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr
820 825 830

Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln
835 840 845

Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val
850 855 860

Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala
865 870 875 880

Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu
885 890 895

Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu
900 905 910

Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr
915 920 925

Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile
930 935 940

Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu
945 950 955 960

Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys
965 970 975

Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala
980 985 990

Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala
995 1000 1005

Ala Ser Gln Ala Ala Leu Gly Leu
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Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val

225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Met Ser Val Phe Asp Ser Lys Phe Lys Gly Ile His Val Tyr Ser
610 615 620

Glu Ile Gly Glu Leu Glu Ser Val Leu Val His Glu Pro Gly Arg Glu
625 630 635 640

Ile Asp Tyr Ile Thr Pro Ala Arg Leu Asp Glu Leu Leu Phe Ser Ala
645 650 655

Ile Leu Glu Ser His Asp Ala Arg Lys Glu His Lys Gln Phe Val Ala
660 665 670

Glu Leu Lys Ala Asn Asp Ile Asn Val Val Glu Leu Ile Asp Leu Val
675 680 685

Ala Glu Thr Tyr Asp Leu Ala Ser Gln Glu Ala Lys Asp Lys Leu Ile
690 695 700

Glu Glu Phe Leu Glu Asp Ser Glu Pro Val Leu Ser Glu Glu His Lys
705 710 715 720

Val Val Val Arg Asn Phe Leu Lys Ala Lys Lys Thr Ser Arg Lys Leu
725 730 735

Val Glu Ile Met Met Ala Gly Ile Thr Lys Tyr Asp Leu Gly Ile Glu
740 745 750

Ala Asp His Glu Leu Ile Val Asp Pro Met Pro Asn Leu Tyr Phe Thr
755 760 765

Arg Asp Pro Phe Ala Ser Val Gly Asn Gly Val Thr Ile His Tyr Met
770 775 780

Arg Tyr Lys Val Arg Gln Arg Glu Thr Leu Phe Ser Arg Phe Val Phe
785 790 795 800

Ser Asn His Pro Lys Leu Ile Asn Thr Pro Trp Tyr Tyr Asp Pro Ser
805 810 815

Leu Lys Leu Ser Ile Glu Gly Gly Asp Val Phe Ile Tyr Asn Asn Asp
820 825 830

Thr Leu Val Val Gly Val Ser Glu Arg Thr Asp Leu Gln Thr Val Thr
835 840 845

Leu Leu Ala Lys Asn Ile Val Ala Asn Lys Glu Cys Glu Phe Lys Arg
850 855 860

Ile Val Ala Ile Asn Val Pro Lys Trp Thr Asn Leu Met His Leu Asp
865 870 875 880

Thr Trp Leu Thr Met Leu Asp Lys Asp Lys Phe Leu Tyr Ser Pro Ile
885 890 895

Ala Asn Asp Val Phe Lys Phe Trp Asp Tyr Asp Leu Val Asn Gly Gly
900 905 910

Ala Glu Pro Gln Pro Val Glu Asn Gly Leu Pro Leu Glu Gly Leu Leu
915 920 925

Gln Ser Ile Ile Asn Lys Lys Pro Val Leu Ile Pro Ile Ala Gly Glu
930 935 940

Gly Ala Ser Gln Met Glu Ile Glu Arg Glu Thr His Phe Asp Gly Thr
945 950 955 960

Asn Tyr Leu Ala Ile Arg Pro Gly Val Val Ile Gly Tyr Ser Arg Asn
965 970 975

Glu Lys Thr Asn Ala Ala Leu Glu Ala Ala Gly Ile Lys Val Leu Pro
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Phe His Gly Asn Gln Leu Ser Leu Gly Met Gly Asn Ala Arg Cys Met
995 1000 1005

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Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
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His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

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Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145                 150                 155                 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
                165                 170                 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
            180                 185                 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
        195                 200                 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
    210                 215                 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225                 230                 235                 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
                245                 250                 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
            260                 265                 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
        275                 280                 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
    290                 295                 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305                 310                 315                 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
                325                 330                 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
            340                 345                 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
        355                 360                 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
    370                 375                 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385                 390                 395                 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
                405                 410                 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
            420                 425                 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
        435                 440                 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
    450                 455                 460
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Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Met Ser Val Phe Asp Ser Lys Phe Lys Gly Ile His Val Tyr Ser
610 615 620

Glu Ile Gly Glu Leu Glu Ser Val Leu Val His Glu Pro Gly Arg Glu
625 630 635 640

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645 650 655

Ile Leu Glu Ser His Asp Ala Arg Lys Glu His Lys Gln Phe Val Ala
660 665 670

Glu Leu Lys Ala Asn Asp Ile Asn Val Val Glu Leu Ile Asp Leu Val
675 680 685

Ala Glu Thr Tyr Asp Leu Ala Ser Gln Glu Ala Lys Asp Lys Leu Ile
690 695 700

Glu Glu Phe Leu Glu Asp Ser Glu Pro Val Leu Ser Glu Glu His Lys
705 710 715 720

Val Val Val Arg Asn Phe Leu Lys Ala Lys Lys Thr Ser Arg Lys Leu
725 730 735

Val Glu Ile Met Met Ala Gly Ile Thr Lys Tyr Asp Leu Gly Ile Glu
740 745 750

Ala Asp His Glu Leu Ile Val Asp Pro Met Pro Asn Leu Tyr Phe Thr
755 760 765

Arg Asp Pro Phe Ala Ser Val Gly Asn Gly Val Thr Ile His Tyr Met
770 775 780

Arg Tyr Lys Val Arg Gln Arg Glu Thr Leu Phe Ser Arg Phe Val Phe

785 790 795 800

Ser Asn His Pro Lys Leu Ile Asn Thr Pro Trp Tyr Tyr Asp Pro Ser
805 810 815

Leu Lys Leu Ser Ile Glu Gly Gly Asp Val Phe Ile Tyr Asn Asn Asp
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Thr Leu Val Val Gly Val Ser Glu Arg Thr Asp Leu Gln Thr Val Thr
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Leu Leu Ala Lys Asn Ile Val Ala Asn Lys Glu Cys Glu Phe Lys Arg
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Ile Val Ala Ile Asn Val Pro Lys Trp Thr Asn Leu Met His Leu Asp
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Thr Trp Leu Thr Met Leu Asp Lys Asp Lys Phe Leu Tyr Ser Pro Ile
885 890 895

Ala Asn Asp Val Phe Lys Phe Trp Asp Tyr Asp Leu Val Asn Gly Gly
900 905 910

Ala Glu Pro Gln Pro Val Glu Asn Gly Leu Pro Leu Glu Gly Leu Leu
915 920 925

Gln Ser Ile Ile Asn Lys Lys Pro Val Leu Ile Pro Ile Ala Gly Glu
930 935 940

Gly Ala Ser Gln Met Glu Ile Glu Arg Glu Thr His Phe Asp Gly Thr
945 950 955 960

Asn Tyr Leu Ala Ile Arg Pro Gly Val Val Ile Gly Tyr Ser Arg Asn
965 970 975

Glu Lys Thr Asn Ala Ala Leu Glu Ala Ala Gly Ile Lys Val Leu Pro
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35 40 45

Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys Leu Gly Cys
50 55 60

Ala Ala Ala Pro Pro Ala Pro Phe Asp Ala His Lys Ser Glu Val Ala
65 70 75 80

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
85 90 95

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
100 105 110

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
115 120 125

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
130 135 140

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
145 150 155 160

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
165 170 175

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
180 185 190

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
195 200 205

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
210 215 220

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
225 230 235 240

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
245 250 255

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
260 265 270

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
275 280 285

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
290 295 300

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
305 310 315 320

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
325 330 335

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
340 345 350

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
355 360 365

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser

370 375 380

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
385 390 395 400

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
405 410 415

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
420 425 430

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465 470 475 480

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
485 490 495

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Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
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Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
545 550 555 560

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
565 570 575

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580 585 590

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
595 600 605

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
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Ser Phe Asp Pro Pro Thr Phe Pro Ala Leu Gly Thr Phe Ser Arg Tyr
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Glu Ser Thr Arg Ser Gly Arg Arg Met Glu Leu Ser Met Gly Pro Ile
65 70 75 80

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85 90 95

Gln Lys Phe Gln Lys Val Lys Gly Phe Gly Gly Ala Met Thr Asp Ala
100 105 110

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115 120 125

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130 135 140

Val Pro Met Ala Ser Cys Asp Phe Ser Ile Arg Thr Tyr Thr Tyr Ala
145 150 155 160

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165 170 175

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180 185 190

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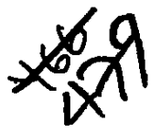
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Val Val Val Val Leu Asn Arg Ser Ser Lys Asp Val Pro Leu Thr Ile
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Lys Asp Pro Ala Val Gly Phe Leu Glu Thr Ile Ser Pro Gly Tyr Ser
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Ile His Thr Tyr Leu Trp Arg Arg Gln Asp Ala His Lys Ser Glu Val
515 520 525

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Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu
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Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu

645 650 655

Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala
660 665 670

Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu
675 680 685

Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp
690 695 700

Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys
705 710 715 720

Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala
725 730 735

Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val
740 745 750

Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His
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Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr
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Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu
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Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val
850 855 860

Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys
865 870 875 880

Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp
885 890 895

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Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu
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Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu
930 935 940

Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys
945 950 955 960

His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val
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Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp
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Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys
995 1000 1005

Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn
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1060 1065 1070

Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys
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Ser Ser Val Val Cys Val Cys Asn Ala Thr Tyr Cys Asp Ser Phe Asp
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Pro Pro Thr Phe Pro Ala Leu Gly Thr Phe Ser Arg Tyr Glu Ser Thr
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Arg Ser Gly Arg Arg Met Glu Leu Ser Met Gly Pro Ile Gln Ala Asn
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His Thr Gly Thr Gly Leu Leu Leu Thr Leu Gln Pro Glu Gln Lys Phe
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Gln Lys Val Lys Gly Phe Gly Gly Ala Met Thr Asp Ala Ala Ala Leu
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Asn Ile Leu Ala Leu Ser Pro Pro Ala Gln Asn Leu Leu Leu Lys Ser
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Tyr Phe Ser Glu Glu Gly Ile Gly Tyr Asn Ile Ile Arg Val Pro Met

130 135 140

Ala Ser Cys Asp Phe Ser Ile Arg Thr Tyr Thr Tyr Ala Asp Thr Pro
145 150 155 160

Asp Asp Phe Gln Leu His Asn Phe Ser Leu Pro Glu Glu Asp Thr Lys
165 170 175

Leu Lys Ile Pro Leu Ile His Arg Ala Leu Gln Leu Ala Gln Arg Pro
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Val Ser Leu Leu Ala Ser Pro Trp Thr Ser Pro Thr Trp Leu Lys Thr
195 200 205

Asn Gly Ala Val Asn Gly Lys Gly Ser Leu Lys Gly Gln Pro Gly Asp
210 215 220

Ile Tyr His Gln Thr Trp Ala Arg Tyr Phe Val Lys Phe Leu Asp Ala
225 230 235 240

Tyr Ala Glu His Lys Leu Gln Phe Trp Ala Val Thr Ala Glu Asn Glu
245 250 255

Pro Ser Ala Gly Leu Leu Ser Gly Tyr Pro Phe Gln Cys Leu Gly Phe
260 265 270

Thr Pro Glu His Gln Arg Asp Phe Ile Ala Arg Asp Leu Gly Pro Thr
275 280 285

Leu Ala Asn Ser Thr His His Asn Val Arg Leu Leu Met Leu Asp Asp
290 295 300

Gln Arg Leu Leu Leu Pro His Trp Ala Lys Val Val Leu Thr Asp Pro
305 310 315 320

Glu Ala Ala Lys Tyr Val His Gly Ile Ala Val His Trp Tyr Leu Asp
325 330 335

Phe Leu Ala Pro Ala Lys Ala Thr Leu Gly Glu Thr His Arg Leu Phe
340 345 350

Pro Asn Thr Met Leu Phe Ala Ser Glu Ala Cys Val Gly Ser Lys Phe
355 360 365

Trp Glu Gln Ser Val Arg Leu Gly Ser Trp Asp Arg Gly Met Gln Tyr
370 375 380

Ser His Ser Ile Ile Thr Asn Leu Leu Tyr His Val Val Gly Trp Thr
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Asp Trp Asn Leu Ala Leu Asn Pro Glu Gly Gly Pro Asn Trp Val Arg
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Asn Phe Val Asp Ser Pro Ile Ile Val Asp Ile Thr Lys Asp Thr Phe
420 425 430

Tyr Lys Gln Pro Met Phe Tyr His Leu Gly His Phe Ser Lys Phe Ile
435 440 445

Pro Glu Gly Ser Gln Arg Val Gly Leu Val Ala Ser Gln Lys Asn Asp
450 455 460

Leu Asp Ala Val Ala Leu Met His Pro Asp Gly Ser Ala Val Val Val
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Val Leu Asn Arg Ser Ser Lys Asp Val Pro Leu Thr Ile Lys Asp Pro
485 490 495

Ala Val Gly Phe Leu Glu Thr Ile Ser Pro Gly Tyr Ser Ile His Thr
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Tyr Leu Trp Arg Arg Gln Asp Ala His Lys Ser Glu Val Ala His Arg
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Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser
565 570 575

Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu
580 585 590

Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys
595 600 605

Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys
610 615 620

Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val
625 630 635 640

Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr
645 650 655

Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu
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Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln
675 680 685

Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg
690 695 700

Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser
705 710 715 720

Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg
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Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu
740 745 750

Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu
755 760 765

Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu
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Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro
785 790 795 800

Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met
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Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe
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Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu
850 855 860

Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala
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Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys
885 890 895

Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu
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Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg
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930 935 940

Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu
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Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn
965 970 975

Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr
980 985 990

Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala
995 1000 1005

Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr
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Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe
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Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
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Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
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Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

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675 680 685

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Leu Ser Pro Pro Ala Gln Asn Leu Leu Leu Lys Ser Tyr Phe Ser Glu
705 710 715 720

Glu Gly Ile Gly Tyr Asn Ile Ile Arg Val Pro Met Ala Ser Cys Asp
725 730 735

Phe Ser Ile Arg Thr Tyr Thr Tyr Ala Asp Thr Pro Asp Asp Phe Gln
740 745 750

Leu His Asn Phe Ser Leu Pro Glu Glu Asp Thr Lys Leu Lys Ile Pro
755 760 765

Leu Ile His Arg Ala Leu Gln Leu Ala Gln Arg Pro Val Ser Leu Leu
770 775 780

Ala Ser Pro Trp Thr Ser Pro Thr Trp Leu Lys Thr Asn Gly Ala Val
785 790 795 800

Asn Gly Lys Gly Ser Leu Lys Gly Gln Pro Gly Asp Ile Tyr His Gln
805 810 815

Thr Trp Ala Arg Tyr Phe Val Lys Phe Leu Asp Ala Tyr Ala Glu His
820 825 830

Lys Leu Gln Phe Trp Ala Val Thr Ala Glu Asn Glu Pro Ser Ala Gly
835 840 845

Leu Leu Ser Gly Tyr Pro Phe Gln Cys Leu Gly Phe Thr Pro Glu His
850 855 860

Gln Arg Asp Phe Ile Ala Arg Asp Leu Gly Pro Thr Leu Ala Asn Ser
865 870 875 880

Thr His His Asn Val Arg Leu Leu Met Leu Asp Asp Gln Arg Leu Leu
885 890 895

Leu Pro His Trp Ala Lys Val Val Leu Thr Asp Pro Glu Ala Ala Lys
900 905 910

Tyr Val His Gly Ile Ala Val His Trp Tyr Leu Asp Phe Leu Ala Pro
915 920 925

Ala Lys Ala Thr Leu Gly Glu Thr His Arg Leu Phe Pro Asn Thr Met
930 935 940

Leu Phe Ala Ser Glu Ala Cys Val Gly Ser Lys Phe Trp Glu Gln Ser
945 950 955 960

Val Arg Leu Gly Ser Trp Asp Arg Gly Met Gln Tyr Ser His Ser Ile
965 970 975

Ile Thr Asn Leu Leu Tyr His Val Val Gly Trp Thr Asp Trp Asn Leu
980 985 990

Ala Leu Asn Pro Glu Gly Gly Pro Asn Trp Val Arg Asn Phe Val Asp
995 1000 1005

Ser Pro Ile Ile Val Asp Ile Thr Lys Asp Thr Phe Tyr Lys Gln Pro
1010 1015 1020

Met Phe Tyr His Leu Gly His Phe Ser Lys Phe Ile Pro Glu Gly Ser
1025 1030 1035 1040

Gln Arg Val Gly Leu Val Ala Ser Gln Lys Asn Asp Leu Asp Ala Val
1045 1050 1055

Ala Leu Met His Pro Asp Gly Ser Ala Val Val Val Val Leu Asn Arg
1060 1065 1070

Ser Ser Lys Asp Val Pro Leu Thr Ile Lys Asp Pro Ala Val Gly Phe
1075 1080 1085

Leu Glu Thr Ile Ser Pro Gly Tyr Ser Ile His Thr Tyr Leu Trp Arg
1090 1095 1100

Arg Gln
1105

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<212> PRT
<213> Homo sapiens

<400> 359
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Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala

100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Ala Arg Pro Cys Ile Pro Lys Ser Phe Gly Tyr Ser Ser Val Val
610 615 620

Cys Val Cys Asn Ala Thr Tyr Cys Asp Ser Phe Asp Pro Pro Thr Phe
625 630 635 640

Pro Ala Leu Gly Thr Phe Ser Arg Tyr Glu Ser Thr Arg Ser Gly Arg
645 650 655

Arg Met Glu Leu Ser Met Gly Pro Ile Gln Ala Asn His Thr Gly Thr
660 665 670

Gly Leu Leu Leu Thr Leu Gln Pro Glu Gln Lys Phe Gln Lys Val Lys
675 680 685

Gly Phe Gly Gly Ala Met Thr Asp Ala Ala Ala Leu Asn Ile Leu Ala
690 695 700

Leu Ser Pro Pro Ala Gln Asn Leu Leu Leu Lys Ser Tyr Phe Ser Glu
705 710 715 720

Glu Gly Ile Gly Tyr Asn Ile Ile Arg Val Pro Met Ala Ser Cys Asp
725 730 735

Phe Ser Ile Arg Thr Tyr Thr Tyr Ala Asp Thr Pro Asp Asp Phe Gln
740 745 750

Leu His Asn Phe Ser Leu Pro Glu Glu Asp Thr Lys Leu Lys Ile Pro
755 760 765

Leu Ile His Arg Ala Leu Gln Leu Ala Gln Arg Pro Val Ser Leu Leu
770 775 780

Ala Ser Pro Trp Thr Ser Pro Thr Trp Leu Lys Thr Asn Gly Ala Val
785 790 795 800

Asn Gly Lys Gly Ser Leu Lys Gly Gln Pro Gly Asp Ile Tyr His Gln
805 810 815

Thr Trp Ala Arg Tyr Phe Val Lys Phe Leu Asp Ala Tyr Ala Glu His
820 825 830

Lys Leu Gln Phe Trp Ala Val Thr Ala Glu Asn Glu Pro Ser Ala Gly
835 840 845

Leu Leu Ser Gly Tyr Pro Phe Gln Cys Leu Gly Phe Thr Pro Glu His
850 855 860

Gln Arg Asp Phe Ile Ala Arg Asp Leu Gly Pro Thr Leu Ala Asn Ser
865 870 875 880

Thr His His Asn Val Arg Leu Leu Met Leu Asp Asp Gln Arg Leu Leu
885 890 895

Leu Pro His Trp Ala Lys Val Val Leu Thr Asp Pro Glu Ala Ala Lys
900 905 910

Tyr Val His Gly Ile Ala Val His Trp Tyr Leu Asp Phe Leu Ala Pro
915 920 925

Ala Lys Ala Thr Leu Gly Glu Thr His Arg Leu Phe Pro Asn Thr Met
930 935 940

Leu Phe Ala Ser Glu Ala Cys Val Gly Ser Lys Phe Trp Glu Gln Ser
945 950 955 960

Val Arg Leu Gly Ser Trp Asp Arg Gly Met Gln Tyr Ser His Ser Ile
965 970 975

Ile Thr Asn Leu Leu Tyr His Val Val Gly Trp Thr Asp Trp Asn Leu
980 985 990

Ala Leu Asn Pro Glu Gly Gly Pro Asn Trp Val Arg Asn Phe Val Asp
995 1000 1005

Ser Pro Ile Ile Val Asp Ile Thr Lys Asp Thr Phe Tyr Lys Gln Pro
1010 1015 1020

Met Phe Tyr His Leu Gly His Phe Ser Lys Phe Ile Pro Glu Gly Ser
1025 1030 1035 1040

Gln Arg Val Gly Leu Val Ala Ser Gln Lys Asn Asp Leu Asp Ala Val
1045 1050 1055

Ala Leu Met His Pro Asp Gly Ser Ala Val Val Val Val Leu Asn Arg
1060 1065 1070

Ser Ser Lys Asp Val Pro Leu Thr Ile Lys Asp Pro Ala Val Gly Phe

1075 1080 1085

Leu Glu Thr Ile Ser Pro Gly Tyr Ser Ile His Thr Tyr Leu Trp Arg
1090 1095 1100

Arg Gln
1105

<210> 360
<211> 661
<212> PRT
<213> Homo sapiens

<400> 360
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Tyr Arg Gln Ser Met Asn Asn Phe
20 25 30

Gln Gly Leu Arg Ser Phe Gly Cys Arg Phe Gly Thr Cys Thr Val Gln
35 40 45

Lys Leu Ala His Gln Ile Tyr Gln Phe Thr Asp Lys Asp Lys Asp Asn
50 55 60

Val Ala Pro Arg Ser Lys Ile Ser Pro Gln Gly Tyr Asp Ala His Lys
65 70 75 80

Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys
85 90 95

Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe
100 105 110

Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr
115 120 125

Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr
130 135 140

Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr
145 150 155 160

Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu
165 170 175

Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val
180 185 190

Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu
195 200 205

Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr
210 215 220

Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala
225 230 235 240

Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro
245 250 255

Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln
260 265 270

Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys
275 280 285

Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe
290 295 300

Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu
305 310 315 320

Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu
325 330 335

Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys
340 345 350

Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu
355 360 365

Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp
370 375 380

Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp
385 390 395 400

Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp
405 410 415

Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr
420 425 430

Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys
435 440 445

Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile
450 455 460

Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln
465 470 475 480

Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr
485 490 495

Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys
500 505 510

Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr
515 520 525

Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro
530 535 540

Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg
545 550 555 560

Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys

565 570 575

Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu
580 585 590

Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu
595 600 605

Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met
610 615 620

Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys
625 630 635 640

Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln
645 650 655

Ala Ala Leu Gly Leu
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<210> 361
<211> 658
<212> PRT
<213> Homo sapiens

<400> 361
Met Trp Trp Arg Leu Trp Trp Leu Leu Leu Leu Leu Leu Leu Leu Trp
1 5 10 15

Pro Met Val Trp Ala Tyr Arg Gln Ser Met Asn Asn Phe Gln Gly Leu
20 25 30

Arg Ser Phe Gly Cys Arg Phe Gly Thr Cys Thr Val Gln Lys Leu Ala
35 40 45

His Gln Ile Tyr Gln Phe Thr Asp Lys Asp Lys Asp Asn Val Ala Pro
50 55 60

Arg Ser Lys Ile Ser Pro Gln Gly Tyr Asp Ala His Lys Ser Glu Val
65 70 75 80

Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val
85 90 95

Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His
100 105 110

Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala
115 120 125

Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly
130 135 140

Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met
145 150 155 160

Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu
165 170 175

Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu
180 185 190

Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu
195 200 205

Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala
210 215 220

Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu
225 230 235 240

Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp
245 250 255

Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys
260 265 270

Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala
275 280 285

Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val
290 295 300

Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His
305 310 315 320

Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr
325 330 335

Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys
340 345 350

Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn
355 360 365

Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu
370 375 380

Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu
385 390 395 400

Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val
405 410 415

Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys
420 425 430

Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp
435 440 445

Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn
450 455 460

Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu
465 470 475 480

Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu
485 490 495

Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys

500 505 510

His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val
515 520 525

Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp
530 535 540

Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys
545 550 555 560

Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn
565 570 575

Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys
580 585 590

Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His
595 600 605

Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe
610 615 620

Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys
625 630 635 640

Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu
645 650 655

Gly Leu

<210> 362
<211> 661
<212> PRT
<213> Homo sapiens

<400> 362
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Tyr Arg Gln Ser Met Asn Asn Phe Gln Gly Leu Arg Ser Phe Gly
610 615 620

Cys Arg Phe Gly Thr Cys Thr Val Gln Lys Leu Ala His Gln Ile Tyr
625 630 635 640

Gln Phe Thr Asp Lys Asp Lys Asp Asn Val Ala Pro Arg Ser Lys Ile
645 650 655

Ser Pro Gln Gly Tyr
660

<210> 363
<211> 661
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<213> Homo sapiens

<400> 363
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Tyr Arg Gln Ser Met Asn Asn Phe Gln Gly Leu Arg Ser Phe Gly
610 615 620

Cys Arg Phe Gly Thr Cys Thr Val Gln Lys Leu Ala His Gln Ile Tyr
625 630 635 640

Gln Phe Thr Asp Lys Asp Lys Asp Asn Val Ala Pro Arg Ser Lys Ile
645 650 655

Ser Pro Gln Gly Tyr
660

<210> 364
<211> 735
<212> PRT
<213> Homo sapiens

<400> 364

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Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
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Tyr Ser Arg Gly Val Phe Arg Arg Asp Ala His Lys Ser Glu Val Ala
            20                  25                  30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
        35                  40                  45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
    50                  55                  60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65                  70                  75                  80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
                85                  90                  95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
            100                 105                 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
        115                 120                 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
    130                 135                 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145                 150                 155                 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
                165                 170                 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
            180                 185                 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
        195                 200                 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
    210                 215                 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225                 230                 235                 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
                245                 250                 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
            260                 265                 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
        275                 280                 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
    290                 295                 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305                 310                 315                 320
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Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Glu Pro Leu Glu Lys Val Ala Ser Val Gly Asn Ser Arg Pro Thr
610 615 620

Gly Gln Gln Leu Glu Ser Leu Gly Leu Leu Ala Pro Gly Glu Gln Ser
625 630 635 640

Leu Pro Cys Thr Glu Arg Lys Pro Ala Ala Thr Ala Arg Leu Ser Arg

645 650 655

Arg Gly Thr Ser Leu Ser Pro Pro Pro Glu Ser Ser Gly Ser Pro Gln
660 665 670

Gln Pro Gly Leu Ser Ala Pro His Ser Arg Gln Ile Pro Ala Pro Gln
675 680 685

Gly Ala Val Leu Val Gln Arg Glu Lys Asp Leu Pro Asn Tyr Asn Trp
690 695 700

Asn Ser Phe Gly Leu Arg Phe Gly Lys Arg Glu Ala Ala Pro Gly Asn
705 710 715 720

His Gly Arg Ser Ala Gly Arg Gly Trp Gly Ala Gly Ala Gly Gln
725 730 735

<210> 365
<211> 735
<212> PRT
<213> Homo sapiens

<400> 365
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190



Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala

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        515                 520                 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
    530                 535                 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545                 550                 555                 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
                565                 570                 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
            580                 585                 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
        595                 600                 605

Leu Glu Pro Leu Glu Lys Val Ala Ser Val Gly Asn Ser Arg Pro Thr
    610                 615                 620

Gly Gln Gln Leu Glu Ser Leu Gly Leu Leu Ala Pro Gly Glu Gln Ser
625                 630                 635                 640

Leu Pro Cys Thr Glu Arg Lys Pro Ala Ala Thr Ala Arg Leu Ser Arg
                645                 650                 655

Arg Gly Thr Ser Leu Ser Pro Pro Pro Glu Ser Ser Gly Ser Pro Gln
            660                 665                 670

Gln Pro Gly Leu Ser Ala Pro His Ser Arg Gln Ile Pro Ala Pro Gln
        675                 680                 685

Gly Ala Val Leu Val Gln Arg Glu Lys Asp Leu Pro Asn Tyr Asn Trp
    690                 695                 700         t

Asn Ser Phe Gly Leu Arg Phe Gly Lys Arg Glu Ala Ala Pro Gly Asn
705                 710                 715                 720

His Gly Arg Ser Ala Gly Arg Gly Trp Gly Ala Gly Ala Gly Gln
                725                 730                 735
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<210> 366
<211> 809
<212> PRT
<213> Homo sapiens

<400> 366

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Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
  1               5                  10                  15

Tyr Ser Arg Ser Leu Asp Lys Arg Lys Asn Leu Glu Pro Val Ser Trp
             20                  25                  30

Ser Ser Leu Asn Pro Lys Phe Leu Ser Gly Lys Gly Leu Val Ile Tyr
         35                  40                  45

Pro Lys Ile Gly Asp Lys Leu Asp Ile Ile Cys Pro Arg Ala Glu Ala
     50                  55                  60
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Gly Arg Pro Tyr Glu Tyr Tyr Lys Leu Tyr Leu Val Arg Pro Glu Gln
65 70 75 80

Ala Ala Ala Cys Ser Thr Val Leu Asp Pro Asn Val Leu Val Thr Cys
85 90 95

Asn Arg Pro Glu Gln Glu Ile Arg Phe Thr Ile Lys Phe Gln Glu Phe
100 105 110

Ser Pro Asn Tyr Met Gly Leu Glu Phe Lys Lys His His Asp Tyr Tyr
115 120 125

Ile Thr Ser Thr Ser Asn Gly Ser Leu Glu Gly Leu Glu Asn Arg Glu
130 135 140

Gly Gly Val Cys Arg Thr Arg Thr Met Lys Ile Ile Met Lys Val Gly
145 150 155 160

Gln Asp Pro Asn Ala Val Thr Pro Glu Gln Leu Thr Thr Ser Arg Pro
165 170 175

Ser Lys Glu Ala Asp Asn Thr Val Lys Met Ala Thr Gln Ala Pro Gly
180 185 190

Ser Arg Gly Ser Leu Gly Asp Ser Asp Gly Lys His Glu Thr Val Asn
195 200 205

Gln Glu Glu Lys Ser Gly Pro Gly Ala Ser Gly Gly Ser Ser Gly Asp
210 215 220

Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu
225 230 235 240

Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln
245 250 255

Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
260 265 270

Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
275 280 285

Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
290 295 300

Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
305 310 315 320

Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
325 330 335

Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His
340 345 350

Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg
355 360 365

Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg
370 375 380

Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala

385 390 395 400

Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser
405 410 415

Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu
420 425 430

Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro
435 440 445

Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys
450 455 460

Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp
465 470 475 480

Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser
485 490 495

Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His
500 505 510

Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser
515 520 525

Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala
530 535 540

Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg
545 550 555 560

Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr
565 570 575

Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu
580 585 590

Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro
595 600 605

Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu
610 615 620

Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro
625 630 635 640

Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys
645 650 655

Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys
660 665 670

Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His
675 680 685

Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser
690 695 700

Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr
705 710 715 720

Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp
725 730 735

Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala
740 745 750

Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu
755 760 765

Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys
770 775 780

Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val
785 790 795 800

Ala Ala Ser Gln Ala Ala Leu Gly Leu
805

<210> 367
<211> 814
<212> PRT
<213> Homo sapiens

<400> 367
Met Ala Arg Pro Gly Gln Arg Trp Leu Gly Lys Trp Leu Val Ala Met
1 5 10 15

Val Val Trp Ala Leu Cys Arg Leu Ala Thr Pro Leu Ala Lys Asn Leu
20 25 30

Glu Pro Val Ser Trp Ser Ser Leu Asn Pro Lys Phe Leu Ser Gly Lys
35 40 45

Gly Leu Val Ile Tyr Pro Lys Ile Gly Asp Lys Leu Asp Ile Ile Cys
50 55 60

Pro Arg Ala Glu Ala Gly Arg Pro Tyr Glu Tyr Tyr Lys Leu Tyr Leu
65 70 75 80

Val Arg Pro Glu Gln Ala Ala Ala Cys Ser Thr Val Leu Asp Pro Asn
85 90 95

Val Leu Val Thr Cys Asn Arg Pro Glu Gln Glu Ile Arg Phe Thr Ile
100 105 110

Lys Phe Gln Glu Phe Ser Pro Asn Tyr Met Gly Leu Glu Phe Lys Lys
115 120 125

His His Asp Tyr Tyr Ile Thr Ser Thr Ser Asn Gly Ser Leu Glu Gly
130 135 140

Leu Glu Asn Arg Glu Gly Gly Val Cys Arg Thr Arg Thr Met Lys Ile
145 150 155 160

Ile Met Lys Val Gly Gln Asp Pro Asn Ala Val Thr Pro Glu Gln Leu
165 170 175

Thr Thr Ser Arg Pro Ser Lys Glu Ala Asp Asn Thr Val Lys Met Ala

180 185 190

Thr Gln Ala Pro Gly Ser Arg Gly Ser Leu Gly Asp Ser Asp Gly Lys
195 200 205

His Glu Thr Val Asn Gln Glu Glu Lys Ser Gly Pro Gly Ala Ser Gly
210 215 220

Gly Ser Ser Gly Asp Asp Ala His Lys Ser Glu Val Ala His Arg Phe
225 230 235 240

Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe
245 250 255

Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val
260 265 270

Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala
275 280 285

Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys
290 295 300

Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys
305 310 315 320

Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp
325 330 335

Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met
340 345 350

Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu
355 360 365

Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu
370 375 380

Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala
385 390 395 400

Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp
405 410 415

Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu
420 425 430

Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu
435 440 445

Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val
450 455 460

Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu
465 470 475 480

Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn
485 490 495

Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu
500 505 510

Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro
515 520 525

Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val
530 535 540

Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu
545 550 555 560

Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu
565 570 575

Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala
580 585 590

Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro
595 600 605

Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe
610 615 620

Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr
625 630 635 640

Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser
645 650 655

Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala
660 665 670

Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln
675 680 685

Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys
690 695 700

Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu
705 710 715 720

Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe
725 730 735

Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile
740 745 750

Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala
755 760 765

Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val
770 775 780

Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu
785 790 795 800

Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
805 810

<210> 368

<211> 660
<212> PRT
<213> Homo sapiens

<400> 368
Met Ala Arg Gly Ser Leu Arg Arg Leu Leu Arg Leu Leu Val Leu Gly
1 5 10 15

Leu Trp Leu Ala Leu Leu Arg Ser Val Ala Gly Glu Gln Ala Pro Gly
20 25 30

Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
35 40 45

Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
50 55 60

Leu Gly Cys Ala Ala Ala Pro Pro Ala Pro Phe Asp Ala His Lys Ser
65 70 75 80

Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala
85 90 95

Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu
100 105 110

Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys
115 120 125

Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu
130 135 140

Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly
145 150 155 160

Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys
165 170 175

Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg
180 185 190

Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr
195 200 205

Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe
210 215 220

Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe
225 230 235 240

Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys
245 250 255

Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg
260 265 270

Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala
275 280 285

Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala
290 295 300

Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys
305 310 315 320

Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala
325 330 335

Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu
340 345 350

Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val
355 360 365

Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe
370 375 380

Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val
385 390 395 400

Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr
405 410 415

Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu
420 425 430

Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val
435 440 445

Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys
450 455 460

Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn
465 470 475 480

Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro
485 490 495

Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys
500 505 510

Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu
515 520 525

Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val
530 535 540

Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg
545 550 555 560

Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu
565 570 575

Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser
580 585 590

Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val
595 600 605

Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp
610 615 620

Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu
625 630 635 640

Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala
645 650 655

Ala Leu Gly Leu
660

<210> 369
<211> 832
<212> PRT
<213> Homo sapiens

<400> 369
Met Leu Arg Arg Arg Gly Ser Pro Gly Met Gly Val His Val Gly Ala
1 5 10 15

Ala Leu Gly Ala Leu Trp Phe Cys Leu Thr Gly Ala Leu Glu Val Gln
20 25 30

Val Pro Glu Asp Pro Val Val Ala Leu Val Gly Thr Asp Ala Thr Leu
35 40 45

Cys Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn
50 55 60

Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Ala
65 70 75 80

Glu Gly Gln Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe
85 90 95

Leu Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val
100 105 110

Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe Val Ser Ile Arg Asp
115 120 125

Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys
130 135 140

Pro Ser Met Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr
145 150 155 160

Val Thr Ile Thr Cys Ser Ser Tyr Arg Gly Tyr Pro Glu Ala Glu Val
165 170 175

Phe Trp Gln Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr
180 185 190

Ser Gln Met Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Val Leu
195 200 205

Arg Val Val Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn
210 215 220

Pro Val Leu Gln Gln Asp Ala His Gly Ser Val Thr Ile Thr Gly Gln
225 230 235 240

Pro Met Thr Phe Pro Pro Glu Asp Ala His Lys Ser Glu Val Ala His
245 250 255

Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile
260 265 270

Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys
275 280 285

Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu
290 295 300

Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys
305 310 315 320

Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp
325 330 335

Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His
340 345 350

Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp
355 360 365

Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys
370 375 380

Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu
385 390 395 400

Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys
405 410 415

Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu
420 425 430

Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala
435 440 445

Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala
450 455 460

Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys
465 470 475 480

Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp
485 490 495

Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys
500 505 510

Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys
515 520 525

Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu
530 535 540

Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys
545 550 555 560

Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met
565 570 575

Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu
580 585 590

Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys
595 600 605

Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe
610 615 620

Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu
625 630 635 640

Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val
645 650 655

Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu
660 665 670

Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro
675 680 685

Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu
690 695 700

Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val
705 710 715 720

Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser
725 730 735

Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu
740 745 750

Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg
755 760 765

Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro
770 775 780

Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala
785 790 795 800

Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala
805 810 815

Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
820 825 830

<210> 370
<211> 809
<212> PRT
<213> Homo sapiens

<400> 370
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Gly Val Phe Arg Arg Asp Ala His Lys Ser Glu Val Ala
20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Lys Asn Leu Glu Pro Val Ser Trp Ser Ser Leu Asn Pro Lys Phe
610 615 620

Leu Ser Gly Lys Gly Leu Val Ile Tyr Pro Lys Ile Gly Asp Lys Leu
625 630 635 640

Asp Ile Ile Cys Pro Arg Ala Glu Ala Gly Arg Pro Tyr Glu Tyr Tyr
645 650 655

Lys Leu Tyr Leu Val Arg Pro Glu Gln Ala Ala Ala Cys Ser Thr Val

660 665 670

Leu Asp Pro Asn Val Leu Val Thr Cys Asn Arg Pro Glu Gln Glu Ile
675 680 685

Arg Phe Thr Ile Lys Phe Gln Glu Phe Ser Pro Asn Tyr Met Gly Leu
690 695 700

Glu Phe Lys Lys His His Asp Tyr Tyr Ile Thr Ser Thr Ser Asn Gly
705 710 715 720

Ser Leu Glu Gly Leu Glu Asn Arg Glu Gly Gly Val Cys Arg Thr Arg
725 730 735

Thr Met Lys Ile Ile Met Lys Val Gly Gln Asp Pro Asn Ala Val Thr
740 745 750

Pro Glu Gln Leu Thr Thr Ser Arg Pro Ser Lys Glu Ala Asp Asn Thr
755 760 765

Val Lys Met Ala Thr Gln Ala Pro Gly Ser Arg Gly Ser Leu Gly Asp
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Gly Ala Ser Gly Gly Ser Ser Gly Asp
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20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His

450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Lys Asn Leu Glu Pro Val Ser Trp Ser Ser Leu Asn Pro Lys Phe
610 615 620

Leu Ser Gly Lys Gly Leu Val Ile Tyr Pro Lys Ile Gly Asp Lys Leu
625 630 635 640

Asp Ile Ile Cys Pro Arg Ala Glu Ala Gly Arg Pro Tyr Glu Tyr Tyr
645 650 655

Lys Leu Tyr Leu Val Arg Pro Glu Gln Ala Ala Ala Cys Ser Thr Val
660 665 670

Leu Asp Pro Asn Val Leu Val Thr Cys Asn Arg Pro Glu Gln Glu Ile
675 680 685

Arg Phe Thr Ile Lys Phe Gln Glu Phe Ser Pro Asn Tyr Met Gly Leu
690 695 700

Glu Phe Lys Lys His His Asp Tyr Tyr Ile Thr Ser Thr Ser Asn Gly
705 710 715 720

Ser Leu Glu Gly Leu Glu Asn Arg Glu Gly Gly Val Cys Arg Thr Arg
725 730 735

Thr Met Lys Ile Ile Met Lys Val Gly Gln Asp Pro Asn Ala Val Thr
740 745 750

Pro Glu Gln Leu Thr Thr Ser Arg Pro Ser Lys Glu Ala Asp Asn Thr
755 760 765

Val Lys Met Ala Thr Gln Ala Pro Gly Ser Arg Gly Ser Leu Gly Asp
770 775 780

Ser Asp Gly Lys His Glu Thr Val Asn Gln Glu Glu Lys Ser Gly Pro
785 790 795 800

Gly Ala Ser Gly Gly Ser Ser Gly Asp
805

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<213> Homo sapiens

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20 25 30

Glu Phe Val Arg Thr Gly Tyr Gly Lys Asp Ile Val Lys Val Leu His
35 40 45

Ile Gln Arg Asp Gly Lys Tyr His Ser Ile Lys Glu Val Ala Thr Ser
50 55 60

Val Gln Leu Thr Leu Ser Ser Lys Lys Asp Tyr Leu His Gly Asp Asn
65 70 75 80

Ser Asp Ile Ile Pro Thr Asp Thr Ile Lys Asn Thr Val His Val Leu
85 90 95

Ala Lys Phe Lys Glu Asn Glu Pro Ala Asn Ile Asp Gly Ala Met Glu
100 105 110

Lys Ala Phe Cys Phe Phe Leu Gln Ile Lys Ser Ile Glu Ala Phe Gly
115 120 125

Val Asn Ile Cys Glu His Phe Leu Ser Ser Phe Asn His Val Ile Arg
130 135 140

Ala Gln Val Tyr Val Glu Glu Ile Pro Trp Lys His Leu Glu Lys Asn
145 150 155 160

Gly Val Lys His Val His Ala Phe Ile His Thr Pro Thr Gly Thr His
165 170 175

Phe Gly Glu Val Glu Gln Leu Arg Ser Gly Pro Gln Val Ile His Ser
180 185 190

Gly Ile Lys Asp Leu Lys Leu Leu Lys Thr Thr Gln Ser Gly Phe Glu
195 200 205

Gly Phe Ile Lys Asp Gln Phe Thr Thr Leu Pro Glu Val Phe Lys Cys
210 215 220

Met Met Arg Thr Leu Pro Gln Ser Ser Phe Pro Leu Phe Gln Val Leu
225 230 235 240

Ser Met Gly Ser Ser Leu Met Asp Thr Ile Arg Asp Leu Val Met Glu

245 250 255

Lys Ser Ala Gly Pro Tyr Asp Lys Asp Glu Tyr Ser Pro Ser Val Gln
260 265 270

Lys Thr Leu Cys Asp Ile Gln Val Leu Ser Leu Ser Arg Val Pro Ala
275 280 285

Ile Glu Asp Met Glu Ile Ser Leu Pro Asn Ile His Tyr Phe Asn Ile
290 295 300

Asp Met Ser Lys Met Gly Leu Ile Asn Lys Glu Glu Val Leu Leu Pro
305 310 315 320

Leu Asp Asn Pro Tyr Gly Lys Ile Thr Gly Thr Val Lys Arg Lys Leu
325 330 335

Ser Ser Arg Leu Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys
340 345 350

Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala
355 360 365

Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn
370 375 380

Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu
385 390 395 400

Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr
405 410 415

Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala
420 425 430

Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp
435 440 445

Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys
450 455 460

Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr
465 470 475 480

Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe
485 490 495

Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala
500 505 510

Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu
515 520 525

Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln
530 535 540

Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser
545 550 555 560

Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr
565 570 575

Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu
580 585 590

Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln
595 600 605

Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu
610 615 620

Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala
625 630 635 640

Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys
645 650 655

Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr
660 665 670

Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg
675 680 685

Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala
690 695 700

Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu
705 710 715 720

Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu
725 730 735

Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr
740 745 750

Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg
755 760 765

Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys
770 775 780

Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu
785 790 795 800

Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys
805 810 815

Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu
820 825 830

Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr
835 840 845

Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys
850 855 860

Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr
865 870 875 880

Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu
885 890 895

Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly
900 905 910

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915 920 925

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20 25 30

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Met Pro Val Phe Ser Leu Gln Asn Asp Glu Val Glu Phe Val Arg
610 615 620

Thr Gly Tyr Gly Lys Asp Ile Val Lys Val Leu His Ile Gln Arg Asp
625 630 635 640

Gly Lys Tyr His Ser Ile Lys Glu Val Ala Thr Ser Val Gln Leu Thr
645 650 655

Leu Ser Ser Lys Lys Asp Tyr Leu His Gly Asp Asn Ser Asp Ile Ile
660 665 670

Pro Thr Asp Thr Ile Lys Asn Thr Val His Val Leu Ala Lys Phe Lys
675 680 685

Glu Asn Glu Pro Ala Asn Ile Asp Gly Ala Met Glu Lys Ala Phe Cys
690 695 700

Phe Phe Leu Gln Ile Lys Ser Ile Glu Ala Phe Gly Val Asn Ile Cys
705 710 715 720

Glu His Phe Leu Ser Ser Phe Asn His Val Ile Arg Ala Gln Val Tyr
725 730 735

Val Glu Glu Ile Pro Trp Lys His Leu Glu Lys Asn Gly Val Lys His
740 745 750

Val His Ala Phe Ile His Thr Pro Thr Gly Thr His Phe Gly Glu Val
755 760 765

Glu Gln Leu Arg Ser Gly Pro Gln Val Ile His Ser Gly Ile Lys Asp
770 775 780

Leu Lys Leu Leu Lys Thr Thr Gln Ser Gly Phe Glu Gly Phe Ile Lys
785 790 795 800

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820 825 830

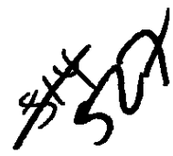
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Glu Ile Ser Leu Pro Asn Ile His Tyr Phe Asn Ile Asp Met Ser Lys
885 890 895

Met Gly Leu Ile Asn Lys Glu Glu Val Leu Leu Pro Leu Asp Asn Pro



900 905 910

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Tyr Thr Glu Met Thr Lys Ser Ser Pro Ser Cys Arg Leu Val Ala Ser
35 40 45

Cys Gln Thr Val Ala Lys Glu Leu Gly Glu Asn Ser Leu Gly Tyr Gly
50 55 60

Pro Gly His Tyr Leu Leu Phe Gly Cys Arg Asp Ala Phe Gly Cys Pro
65 70 75 80

Met Pro Gly Leu Phe His Leu Leu Gln Asp Gln Met Ile Gly Ser Leu
85 90 95

His Thr Asp Ser Leu Pro Asn Asp Glu Val Glu Phe Val Arg Thr Gly
100 105 110

Tyr Gly Lys Glu Met Val Lys Val Leu His Ile Gln Arg Asp Gly Lys
115 120 125

Tyr His Ser Ile Lys Glu Val Ala Thr Ser Val Gln Leu Thr Leu Ser
130 135 140

Ser Lys Lys Asp Tyr Leu His Gly Asp Asn Ser Asp Ile Ile Pro Thr
145 150 155 160

Asp Thr Ile Lys Asn Thr Val His Val Leu Ala Lys Phe Lys Glu Asn
165 170 175

Asp Pro Ala Asn Ile Asp Gly Ala Met Glu Lys Ala Phe Cys Phe Phe
180 185 190

Leu Gln Ile Lys Ser Ile Glu Ala Phe Gly Val Asn Ile Cys Glu His
195 200 205

Phe Leu Ser Ser Phe Asn His Val Ile Arg Ala Gln Val Tyr Met Glu
210 215 220

Glu Ile Pro Trp Lys His Leu Gly Lys Asn Gly Val Lys His Val His
225 230 235 240

Ala Phe Ile His Thr Pro Thr Gly Thr His Phe Cys Glu Val Glu Gln
245 250 255

Leu Arg Ser Gly Pro Gln Val Ile His Ser Gly Ile Lys Asp Leu Lys
260 265 270

Val Leu Lys Thr Thr Gln Ser Gly Phe Glu Gly Phe Ile Lys Asp Gln
275 280 285

Phe Thr Thr Leu Pro Glu Val Lys Asp Arg Cys Phe Ala Thr Gln Val
290 295 300

Tyr Cys Lys Trp Arg Tyr His Gln Cys Arg Asp Val Asp Phe Lys Ala
305 310 315 320

Thr Trp Asp Thr Ile Arg Asp Leu Val Met Glu Lys Ser Ala Gly Pro
325 330 335

Tyr Asp Lys Gly Glu Tyr Leu Thr Ser Val Gln Lys Thr Leu Cys Asp
340 345 350

Ile Gln Val Leu Ser Leu Ser Arg Val Pro Ala Ile Glu Asp Met Glu
355 360 365

Ile Ser Leu Pro Asn Ile His Tyr Phe Asn Ile Asp Met Ser Lys Met
370 375 380

Gly Leu Ile Asn Lys Glu Glu Val Leu Leu Pro Leu Asp Asn Pro Tyr
385 390 395 400

Gly Lys Ile Thr Gly Thr Val Lys Arg Lys Leu Ser Ser Arg Leu Asp
405 410 415

Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu
420 425 430

Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln
435 440 445

Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe
450 455 460

Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser
465 470 475 480

Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg
485 490 495

Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu
500 505 510

Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro
515 520 525

Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp
530 535 540

Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg
545 550 555 560

His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr
565 570 575

Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys

580 585 590

Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser
595 600 605

Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg
610 615 620

Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys
625 630 635 640

Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val
645 650 655

His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg
660 665 670

Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser
675 680 685

Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys
690 695 700

Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu
705 710 715 720

Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu
725 730 735

Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg
740 745 750

His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr
755 760 765

Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys
770 775 780

Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln
785 790 795 800

Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr
805 810 815

Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln
820 825 830

Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val
835 840 845

Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala
850 855 860

Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu
865 870 875 880

Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu
885 890 895

Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr
900 905 910

Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile
915 920 925

Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu
930 935 940

Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys
945 950 955 960

Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala
965 970 975

Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala
980 985 990

Ala Ser Gln Ala Ala Leu Gly Leu
995 1000

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His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
35 40 45

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
50 55 60

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
65 70 75 80

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
85 90 95

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
100 105 110

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
115 120 125

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
130 135 140

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
145 150 155 160

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
165 170 175

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys

180 185 190

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
195 200 205

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
210 215 220

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
225 230 235 240

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
245 250 255

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
260 265 270

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
275 280 285

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
290 295 300

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
305 310 315 320

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
325 330 335

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
340 345 350

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
355 360 365

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
370 375 380

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
385 390 395 400

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
405 410 415

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
420 425 430

Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
435 440 445

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
450 455 460

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
465 470 475 480

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
485 490 495

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
500 505 510

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
515 520 525

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
530 535 540

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
545 550 555 560

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
565 570 575

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

Leu Met Glu Lys Phe Ile Trp Tyr Leu Val His Leu Tyr Thr Glu Met
610 615 620

Thr Lys Ser Ser Pro Ser Cys Arg Leu Val Ala Ser Cys Gln Thr Val
625 630 635 640

Ala Lys Glu Leu Gly Glu Asn Ser Leu Gly Tyr Gly Pro Gly His Tyr
645 650 655

Leu Leu Phe Gly Cys Arg Asp Ala Phe Gly Cys Pro Met Pro Gly Leu
660 665 670

Phe His Leu Leu Gln Asp Gln Met Ile Gly Ser Leu His Thr Asp Ser
675 680 685

Leu Pro Asn Asp Glu Val Glu Phe Val Arg Thr Gly Tyr Gly Lys Glu
690 695 700

Met Val Lys Val Leu His Ile Gln Arg Asp Gly Lys Tyr His Ser Ile
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725 730 735

Tyr Leu His Gly Asp Asn Ser Asp Ile Ile Pro Thr Asp Thr Ile Lys
740 745 750

Asn Thr Val His Val Leu Ala Lys Phe Lys Glu Asn Asp Pro Ala Asn
755 760 765

Ile Asp Gly Ala Met Glu Lys Ala Phe Cys Phe Phe Leu Gln Ile Lys
770 775 780

Ser Ile Glu Ala Phe Gly Val Asn Ile Cys Glu His Phe Leu Ser Ser
785 790 795 800

Phe Asn His Val Ile Arg Ala Gln Val Tyr Met Glu Glu Ile Pro Trp
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850 855 860

Thr Gln Ser Gly Phe Glu Gly Phe Ile Lys Asp Gln Phe Thr Thr Leu
865 870 875 880

Pro Glu Val Lys Asp Arg Cys Phe Ala Thr Gln Val Tyr Cys Lys Trp
885 890 895

Arg Tyr His Gln Cys Arg Asp Val Asp Phe Lys Ala Thr Trp Asp Thr
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915 920 925

Glu Tyr Leu Thr Ser Val Gln Lys Thr Leu Cys Asp Ile Gln Val Leu
930 935 940

Ser Leu Ser Arg Val Pro Ala Ile Glu Asp Met Glu Ile Ser Leu Pro
945 950 955 960

Asn Ile His Tyr Phe Asn Ile Asp Met Ser Lys Met Gly Leu Ile Asn
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35 40 45

Val Leu His Ile Gln Arg Asp Gly Lys Tyr His Ser Ile Lys Glu Val
50 55 60

Ala Thr Ser Val Gln Leu Thr Leu Ser Ser Lys Lys Asp Tyr Leu His
65 70 75 80

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85 90 95

His Val Leu Ala Lys Phe Lys Gly Ile Lys Ser Ile Glu Ala Phe Gly
100 105 110

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115 120 125

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130 135 140

Gly Val Lys His Val His Ala Phe Ile His Thr Pro Thr Gly Thr His
145 150 155 160

Phe Cys Glu Val Glu Gln Leu Arg Ser Gly Pro Pro Val Ile His Ser
165 170 175

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180 185 190

Gly Phe Ile Lys Asp Gln Phe Thr Thr Leu Pro Glu Val Lys Asp Arg
195 200 205

Cys Phe Ala Thr Gln Val Tyr Cys Lys Trp Arg Tyr His Gln Cys Arg
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225 230 235 240

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245 250 255

Gln Lys Thr Leu Tyr Asp Ile Gln Val Leu Ser Leu Ser Arg Val Pro
260 265 270

Glu Ile Glu Asp Met Glu Ile Ser Leu Pro Asn Ile His Tyr Phe Asn
275 280 285

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290 295 300

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305 310 315 320

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325 330 335

Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe
340 345 350

Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val Lys Leu Val
355 360 365

Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala
370 375 380

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385 390 395 400

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405 410 415

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435 440 445

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Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu
465 470 475 480

Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala
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Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val
545 550 555 560

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565 570 575

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580 585 590

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625 630 635 640

Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu
645 650 655

Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu
660 665 670

Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala
675 680 685

Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro
690 695 700

Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe
705 710 715 720

Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr
725 730 735

Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser
740 745 750

Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala
755 760 765

Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln
770 775 780

Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys
785 790 795 800

Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu
805 810 815

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820 825 830

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835 840 845

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850 855 860

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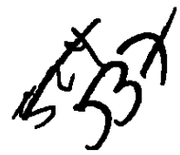
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Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
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Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
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Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
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580 585 590

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
595 600 605

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610 615 620

Phe Val Arg Thr Gly Tyr Gly Lys Asp Met Val Lys Val Leu His Ile
625 630 635 640

Gln Arg Asp Gly Lys Tyr His Ser Ile Lys Glu Val Ala Thr Ser Val
645 650 655

Gln Leu Thr Leu Ser Ser Lys Lys Asp Tyr Leu His Gly Asp Asn Ser
660 665 670

Asp Ile Ile Pro Thr Asp Thr Ile Lys Asn Thr Val His Val Leu Ala
675 680 685

Lys Phe Lys Gly Ile Lys Ser Ile Glu Ala Phe Gly Val Asn Ile Cys
690 695 700

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Val Glu Glu Ile Pro Trp Lys Arg Leu Glu Lys Asn Gly Val Lys His
725 730 735

Val His Ala Phe Ile His Thr Pro Thr Gly Thr His Phe Cys Glu Val
740 745 750

Glu Gln Leu Arg Ser Gly Pro Pro Val Ile His Ser Gly Ile Lys Asp
755 760 765

Leu Lys Val Leu Lys Thr Thr Gln Ser Gly Phe Glu Gly Phe Ile Lys
770 775 780

Asp Gln Phe Thr Thr Leu Pro Glu Val Lys Asp Arg Cys Phe Ala Thr

785 790 795 800

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805 810 815

Glu Ala Thr Trp Gly Thr Ile Arg Asp Leu Val Leu Glu Lys Phe Ala
820 825 830

Gly Pro Tyr Asp Lys Gly Glu Tyr Ser Pro Ser Val Gln Lys Thr Leu
835 840 845

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850 855 860

Met Glu Ile Ser Leu Pro Asn Ile His Tyr Phe Asn Ile Asp Met Ser
865 870 875 880

Lys Met Gly Leu Ile Asn Lys Glu Glu Val Leu Leu Pro Leu Asp Asn
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Val Ala Ser Cys Gln Thr Val Ala Lys Glu Leu Gly Glu Asn Ser Leu
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Gly Tyr Gly Pro Gly His Tyr Leu Leu Phe Gly Cys Arg Asp Ala Phe
65 70 75 80

Gly Cys Pro Met Pro Gly Leu Phe His Leu Leu Gln Asp Gln Met Ile
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Gly Ser Leu His Thr Asp Ser Leu Pro Asn Asp Glu Val Glu Phe Val
100 105 110

Arg Thr Gly Tyr Gly Lys Glu Met Val Lys Val Leu His Ile Gln Arg
115 120 125

Asp Gly Lys Tyr His Ser Ile Lys Glu Val Ala Thr Ser Val Gln Leu
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Thr Leu Ser Ser Lys Lys Asp Tyr Leu His Gly Asp Asn Ser Asp Ile

145 150 155 160

Ile Pro Thr Asp Thr Ile Lys Asn Thr Val His Val Leu Ala Lys Phe
165 170 175

Lys Glu Asn Asp Pro Ala Asn Ile Asp Gly Ala Met Glu Lys Ala Phe
180 185 190

Cys Phe Phe Leu Gln Ile Lys Ser Ile Glu Ala Phe Gly Val Asn Ile
195 200 205

Cys Glu His Phe Leu Ser Ser Phe Asn His Val Ile Arg Ala Gln Val
210 215 220

Tyr Met Glu Glu Ile Pro Trp Lys His Leu Gly Lys Asn Gly Val Lys
225 230 235 240

His Val His Ala Phe Ile His Thr Pro Thr Gly Thr His Phe Cys Glu
245 250 255

Val Glu Gln Leu Arg Ser Gly Pro Gln Val Ile His Ser Gly Ile Lys
260 265 270

Asp Leu Lys Val Leu Lys Thr Thr Gln Ser Gly Phe Glu Gly Phe Ile
275 280 285

Lys Asp Gln Phe Thr Thr Leu Pro Glu Val Lys Asp Arg Cys Phe Ala
290 295 300

Thr Gln Val Tyr Cys Lys Trp Arg Tyr His Gln Cys Arg Asp Val Asp
305 310 315 320

Phe Lys Ala Thr Trp Asp Thr Ile Arg Asp Leu Val Met Glu Lys Ser
325 330 335

Ala Gly Pro Tyr Asp Lys Gly Glu Tyr Leu Thr Ser Val Gln Lys Thr
340 345 350

Leu Cys Asp Ile Gln Val Leu Ser Leu Ser Arg Val Pro Ala Ile Glu
355 360 365

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370 375 380

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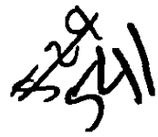
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465 470 475 480



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Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile
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Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala
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Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys
580 585 590

Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys
595 600 605

Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe
610 615 620

Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg
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Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu
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Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala
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675 680 685

Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys
690 695 700

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725 730 735

Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr
740 745 750

Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala
755 760 765

Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro
770 775 780

His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu
785 790 795 800

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820 825 830

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835 840 845

Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met
850 855 860

Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val
865 870 875 880

Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr
885 890 895

Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp
900 905 910

Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His
915 920 925

Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln
930 935 940

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945 950 955 960

Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys
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50 55 60

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65 70 75 80

Gly Asp Asn Ser Asp Ile Ile Pro Thr Asp Thr Ile Lys Asn Thr Val
85 90 95

His Val Leu Ala Lys Phe Lys Glu Asn Glu Pro Ala Asn Ile Asp Gly
100 105 110

Ala Met Glu Lys Ala Phe Cys Phe Phe Leu Gln Ile Lys Ser Ile Glu
115 120 125

Ala Phe Gly Val Asn Ile Cys Glu His Phe Leu Ser Ser Phe Asn His
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180 185 190

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370 375 380

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385 390 395 400

Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp Lys
405 410 415

Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala Asp
420 425 430

Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln His
435 440 445

Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp
450 455 460

Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys
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485 490 495

Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys
500 505 510

Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu
515 520 525

Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala
530 535 540

Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala
545 550 555 560

Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys
565 570 575

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580 585 590

Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys
595 600 605

Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys
610 615 620

Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu
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Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys
645 650 655

Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met
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755 760 765

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785 790 795 800

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865 870 875 880

Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala
885 890 895

Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala
900 905 910

Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
915 920 925

<210> 380
<211> 913
<212> PRT
<213> Homo sapiens

<400> 380
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg Met Ala Asp Tyr His Asn Asn Tyr
20 25 30

Lys Lys Asn Asp Glu Leu Glu Phe Val Arg Thr Gly Tyr Gly Lys Asp
35 40 45

Met Val Lys Val Leu His Ile Gln Arg Asp Gly Lys Tyr His Ser Ile
50 55 60

Lys Glu Val Ala Thr Ser Val Gln Leu Thr Leu Ser Ser Lys Lys Asp
65 70 75 80

Tyr Leu His Gly Asp Asn Ser Asp Ile Ile Pro Thr Asp Thr Ile Lys

85 90 95

Asn Thr Val His Val Leu Ala Lys Phe Lys Gly Ile Lys Ser Ile Glu
100 105 110

Ala Phe Gly Val Asn Ile Cys Glu Tyr Phe Leu Ser Ser Phe Asn His
115 120 125

Val Ile Arg Ala Gln Val Tyr Val Glu Glu Ile Pro Trp Lys Arg Leu
130 135 140

Glu Lys Asn Gly Val Lys His Val His Ala Phe Ile His Thr Pro Thr
145 150 155 160

Gly Thr His Phe Cys Glu Val Glu Gln Leu Arg Ser Gly Pro Pro Val
165 170 175

Ile His Ser Gly Ile Lys Asp Leu Lys Val Leu Lys Thr Thr Gln Ser
180 185 190

Gly Phe Glu Gly Phe Ile Lys Asp Gln Phe Thr Thr Leu Pro Glu Val
195 200 205

Lys Asp Arg Cys Phe Ala Thr Gln Val Tyr Cys Lys Trp Arg Tyr His
210 215 220

Gln Cys Arg Asp Val Asp Phe Glu Ala Thr Trp Gly Thr Ile Arg Asp
225 230 235 240

Leu Val Leu Glu Lys Phe Ala Gly Pro Tyr Asp Lys Gly Glu Tyr Ser
245 250 255

Pro Ser Val Gln Lys Thr Leu Tyr Asp Ile Gln Val Leu Ser Leu Ser
260 265 270

Arg Val Pro Glu Ile Glu Asp Met Glu Ile Ser Leu Pro Asn Ile His
275 280 285

Tyr Phe Asn Ile Asp Met Ser Lys Met Gly Leu Ile Asn Lys Glu Glu
290 295 300

Val Leu Leu Pro Leu Asp Asn Pro Tyr Gly Lys Ile Thr Gly Thr Val
305 310 315 320

Lys Arg Lys Leu Ser Ser Arg Leu Asp Ala His Lys Ser Glu Val Ala
325 330 335

His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe Lys Ala Leu Val Leu
340 345 350

Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro Phe Glu Asp His Val
355 360 365

Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys Thr Cys Val Ala Asp
370 375 380

Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His Thr Leu Phe Gly Asp
385 390 395 400

Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr Tyr Gly Glu Met Ala
405 410 415

Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn Glu Cys Phe Leu Gln
420 425 430

His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val
435 440 445

Asp Val Met Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys
450 455 460

Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro
465 470 475 480

Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys
485 490 495

Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu
500 505 510

Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys
515 520 525

Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val
530 535 540

Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser
545 550 555 560

Lys Leu Val Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly
565 570 575

Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile
580 585 590

Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu
595 600 605

Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp
610 615 620

Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser
625 630 635 640

Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly
645 650 655

Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val
660 665 670

Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys
675 680 685

Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu
690 695 700

Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys
705 710 715 720

Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu
725 730 735



Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val
740 745 750

Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His
755 760 765

Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val
770 775 780

Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg
785 790 795 800

Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe
805 810 815

Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala
820 825 830

Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu
835 840 845

Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys
850 855 860

Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala
865 870 875 880

Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe
885 890 895

Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly
900 905 910

Leu

<210> 381
<211> 805
<212> PRT
<213> Homo sapiens

<400> 381
Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
1 5 10 15

Leu Glu His Thr His Arg Arg Gly Ser Leu Asp Lys Arg Asp Ala His
20 25 30

Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe
35 40 45

Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Cys Pro
50 55 60

Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys
65 70 75 80

Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His
85 90 95

Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr
100 105 110

Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn
115 120 125

Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu
130 135 140

Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu
145 150 155 160

Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro
165 170 175

Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala
180 185 190

Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu
195 200 205

Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys
210 215 220

Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe
225 230 235 240

Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu
245 250 255

Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr
260 265 270

Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp
275 280 285

Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu
290 295 300

Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala
305 310 315 320

Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala
325 330 335

Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys
340 345 350

Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro
355 360 365

Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr
370 375 380

Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala
385 390 395 400

Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu
405 410 415

Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe
420 425 430

Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser
435 440 445

Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser
450 455 460

Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp
465 470 475 480

Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr
485 490 495

Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn
500 505 510

Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro
515 520 525

Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr
530 535 540

Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu
545 550 555 560

Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val
565 570 575

Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp
580 585 590

Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser
595 600 605

Gln Ala Ala Leu Gly Leu Phe Pro Thr Ile Pro Leu Ser Arg Leu Phe
610 615 620

Asp Asn Ala Met Leu Arg Ala His Arg Leu His Gln Leu Ala Phe Asp
625 630 635 640

Thr Tyr Gln Glu Phe Glu Glu Ala Tyr Ile Pro Lys Glu Gln Lys Tyr
645 650 655

Ser Phe Leu Gln Asn Pro Gln Thr Ser Leu Cys Phe Ser Glu Ser Ile
660 665 670

Pro Thr Pro Ser Asn Arg Glu Glu Thr Gln Gln Lys Ser Asn Leu Glu
675 680 685

Leu Leu Arg Ile Ser Leu Leu Leu Ile Gln Ser Trp Leu Glu Pro Val
690 695 700

Gln Phe Leu Arg Ser Val Phe Ala Asn Ser Leu Val Tyr Gly Ala Ser
705 710 715 720

Asp Ser Asn Val Tyr Asp Leu Leu Lys Asp Leu Glu Glu Gly Ile Gln
725 730 735

Thr Leu Met Gly Arg Leu Glu Asp Gly Ser Pro Arg Thr Gly Gln Ile

740 745 750

Phe Lys Gln Thr Tyr Ser Lys Phe Asp Thr Asn Ser His Asn Asp Asp
755 760 765

Ala Leu Leu Lys Asn Tyr Gly Leu Leu Tyr Cys Phe Arg Lys Asp Met
770 775 780

Asp Lys Val Glu Thr Phe Leu Arg Ile Val Gln Cys Arg Ser Val Glu
785 790 795 800

Gly Ser Cys Gly Phe
805

<210> 382
<211> 191
<212> PRT
<213> Homo sapiens

<400> 382
Phe Pro Thr Ile Pro Leu Ser Arg Leu Phe Asp Asn Ala Met Leu Arg
1 5 10 15

Ala His Arg Leu His Gln Leu Ala Phe Asp Thr Tyr Gln Glu Phe Glu
20 25 30

Glu Ala Tyr Ile Pro Lys Glu Gln Lys Tyr Ser Phe Leu Gln Asn Pro
35 40 45

Gln Thr Ser Leu Cys Phe Ser Glu Ser Ile Pro Thr Pro Ser Asn Arg
50 55 60

Glu Glu Thr Gln Gln Lys Ser Asn Leu Glu Leu Leu Arg Ile Ser Leu
65 70 75 80

Leu Leu Ile Gln Ser Trp Leu Glu Pro Val Gln Phe Leu Arg Ser Val
85 90 95

Phe Ala Asn Ser Leu Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr Asp
100 105 110

Leu Leu Lys Asp Leu Glu Glu Gly Ile Gln Thr Leu Met Gly Arg Leu
115 120 125

Glu Asp Gly Ser Pro Arg Thr Gly Gln Ile Phe Lys Gln Thr Tyr Ser
130 135 140

Lys Phe Asp Thr Asn Ser His Asn Asp Asp Ala Leu Leu Lys Asn Tyr
145 150 155 160

Gly Leu Leu Tyr Cys Phe Arg Lys Asp Met Asp Lys Val Glu Thr Phe
165 170 175

Leu Arg Ile Val Gln Cys Arg Ser Val Glu Gly Ser Cys Gly Phe
180 185 190

<210> 383

<211> 191
<212> PRT
<213> Homo sapiens

<400> 383
Phe Pro Thr Ile Pro Leu Ser Arg Leu Phe Asp Asn Ala Met Leu Arg
1 5 10 15

Ala His Arg Leu His Gln Leu Ala Phe Asp Thr Tyr Gln Glu Phe Glu
20 25 30

Glu Ala Tyr Ile Pro Lys Glu Gln Lys Tyr Ser Phe Leu Gln Asn Pro
35 40 45

Gln Thr Ser Leu Cys Phe Ser Glu Ser Ile Pro Thr Pro Ser Asn Arg
50 55 60

Glu Glu Thr Gln Gln Lys Ser Asn Leu Glu Leu Leu Arg Ile Ser Leu
65 70 75 80

Leu Leu Ile Gln Ser Trp Leu Glu Pro Val Gln Phe Leu Arg Ser Val
85 90 95

Phe Ala Asn Ser Leu Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr Asp
100 105 110

Leu Leu Lys Asp Leu Glu Glu Gly Ile Gln Thr Leu Met Gly Arg Leu
115 120 125

Glu Asp Gly Ser Pro Arg Thr Gly Gln Ile Phe Lys Gln Thr Tyr Ser
130 135 140

Lys Phe Asp Thr Asn Ser His Asn Asp Asp Ala Leu Leu Lys Asn Tyr
145 150 155 160

Gly Leu Leu Tyr Cys Phe Arg Lys Asp Met Asp Lys Val Glu Thr Phe
165 170 175

Leu Arg Ile Val Gln Cys Arg Ser Val Glu Gly Ser Cys Gly Phe
180 185 190

<210> 384
<211> 191
<212> PRT
<213> Homo sapiens

<400> 384
Phe Pro Thr Ile Pro Leu Ser Arg Leu Phe Asp Asn Ala Met Leu Arg
1 5 10 15

Ala His Arg Leu His Gln Leu Ala Phe Asp Thr Tyr Gln Glu Phe Glu
20 25 30

Glu Ala Tyr Ile Pro Lys Glu Gln Lys Tyr Ser Phe Leu Gln Asn Pro
35 40 45

Gln Thr Ser Leu Cys Phe Ser Glu Ser Ile Pro Thr Pro Ser Asn Arg
50 55 60

Glu Glu Thr Gln Gln Lys Ser Asn Leu Glu Leu Leu Arg Ile Ser Leu
65 70 75 80

Leu Leu Ile Gln Ser Trp Leu Glu Pro Val Gln Phe Leu Arg Ser Val
85 90 95

Phe Ala Asn Ser Leu Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr Asp
100 105 110

Leu Leu Lys Asp Leu Glu Glu Gly Ile Gln Thr Leu Met Gly Arg Leu
115 120 125

Glu Asp Gly Ser Pro Arg Thr Gly Gln Ile Phe Lys Gln Thr Tyr Ser
130 135 140

Lys Phe Asp Thr Asn Ser His Asn Asp Asp Ala Leu Leu Lys Asn Tyr
145 150 155 160

Gly Leu Leu Tyr Cys Phe Arg Lys Asp Met Asp Lys Val Glu Thr Phe
165 170 175

Leu Arg Ile Val Gln Cys Arg Ser Val Glu Gly Ser Cys Gly Phe
180 185 190

<210> 385
<211> 165
<212> PRT
<213> Homo sapiens

<400> 385
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> 386
<211> 119
<212> PRT
<213> Homo sapiens

<400> 386
His Ser Asp Pro Ala Arg Arg Gly Glu Leu Ser Val Cys Asp Ser Ile
1 5 10 15

Ser Glu Trp Val Thr Ala Ala Asp Lys Lys Thr Ala Val Asp Met Ser
20 25 30

Gly Gly Thr Val Thr Val Leu Glu Lys Val Pro Val Ser Lys Gly Gln
35 40 45

Leu Lys Gln Tyr Phe Tyr Glu Thr Lys Cys Asn Pro Met Gly Tyr Thr
50 55 60

Lys Glu Gly Cys Arg Gly Ile Asp Lys Arg His Trp Asn Ser Gln Cys
65 70 75 80

Arg Thr Thr Gln Ser Tyr Val Arg Ala Leu Thr Met Asp Ser Lys Lys
85 90 95

Arg Ile Gly Trp Arg Phe Ile Arg Ile Asp Thr Ser Cys Val Cys Thr
100 105 110

Leu Thr Ile Lys Arg Gly Arg
115

<210> 387
<211> 60
<212> PRT
<213> Homo sapiens

<400> 387
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg His Gly
20 25 30

Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala
35 40 45

Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg
50 55 60

<210> 388
<211> 32
<212> PRT
<213> Homo sapiens

<400> 388
Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met Asp
1 5 10 15

Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His
20 25 30

<210> 389
<211> 32
<212> PRT
<213> Homo sapiens

<400> 389
Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met Asp
1 5 10 15

Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His
20 25 30

<210> 390
<211> 60
<212> PRT
<213> Homo sapiens

<400> 390
His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg His Gly
20 25 30

Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala
35 40 45

Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg
50 55 60

<210> 391
<211> 29
<212> PRT
<213> Homo sapiens

<400> 391
Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met Asp
1 5 10 15

Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu
20 25

<210> 392
<211> 32
<212> PRT

<213> Homo sapiens

<400> 392
Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met Asp
1 5 10 15

Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His
20 25 30

<210> 393
<211> 32
<212> PRT
<213> Homo sapiens

<400> 393
Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met Asp
1 5 10 15

Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His
20 25 30

<210> 394
<211> 32
<212> PRT
<213> Homo sapiens

<400> 394
Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met Asp
1 5 10 15

Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His
20 25 30

<210> 395
<211> 32
<212> PRT
<213> Homo sapiens

<400> 395
Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met Asp
1 5 10 15

Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His
20 25 30

<210> 396
<211> 32
<212> PRT
<213> Homo sapiens

<400> 396
Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Gly Lys Met Asp
1 5 10 15

Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His
20 25 30

<210> 397
<211> 29
<212> PRT
<213> Homo sapiens

<400> 397
Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met Asp
1 5 10 15

Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu
20 25

<210> 398
<211> 32
<212> PRT
<213> Homo sapiens

<400> 398
Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Gly Lys Met Asp
1 5 10 15

Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His
20 25 30

<210> 399
<211> 32
<212> PRT
<213> Homo sapiens

<400> 399
Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Gly Met Asp
1 5 10 15

Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His
20 25 30

<210> 400
<211> 32
<212> PRT
<213> Homo sapiens

<400> 400
Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met Asp
1 5 10 15

Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His
20 25 30

<210> 401
<211> 30
<212> PRT
<213> Homo sapiens

<400> 401
Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met Asp
1 5 10 15

Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg
20 25 30

<210> 402
<211> 151
<212> PRT
<213> Homo sapiens

<400> 402
Met Ser Ser Phe Ser Thr Thr Thr Val Ser Phe Leu Leu Leu Leu Ala
1 5 10 15

Phe Gln Leu Leu Gly Gln Thr Arg Ala Asn Pro Met Tyr Asn Ala Val
20 25 30

Ser Asn Ala Asp Leu Met Asp Phe Lys Asn Leu Leu Asp His Leu Glu
35 40 45

Glu Lys Met Pro Leu Glu Asp Glu Val Val Pro Pro Gln Val Leu Ser
50 55 60

Glu Pro Asn Glu Glu Ala Gly Ala Ala Leu Ser Pro Leu Pro Glu Val
65 70 75 80

Pro Pro Trp Thr Gly Glu Val Ser Pro Ala Gln Arg Asp Gly Gly Ala
85 90 95

Leu Gly Arg Gly Pro Trp Asp Ser Ser Asp Arg Ser Ala Leu Leu Lys
100 105 110

Ser Lys Leu Arg Ala Leu Leu Thr Ala Pro Arg Ser Leu Arg Arg Ser
115 120 125

Ser Cys Phe Gly Gly Arg Met Asp Arg Ile Gly Ala Gln Ser Gly Leu
130 135 140

Gly Cys Asn Ser Phe Arg Tyr
145 150

<210> 403
<211> 151
<212> PRT
<213> Homo sapiens

<400> 403
Met Ser Ser Phe Ser Thr Thr Thr Val Ser Phe Leu Leu Leu Leu Ala
1 5 10 15

Phe Gln Leu Leu Gly Gln Thr Arg Ala Asn Pro Met Tyr Asn Ala Val
20 25 30

Ser Asn Ala Asp Leu Met Asp Phe Lys Asn Leu Leu Asp His Leu Glu
35 40 45

Glu Lys Met Pro Leu Glu Asp Glu Val Val Pro Pro Gln Val Leu Ser
50 55 60

Glu Pro Asn Glu Glu Ala Gly Ala Ala Leu Ser Pro Leu Pro Glu Val
65 70 75 80

Pro Pro Trp Thr Gly Glu Val Ser Pro Ala Gln Arg Asp Gly Gly Ala
85 90 95

Leu Gly Arg Gly Pro Trp Asp Ser Ser Asp Arg Ser Ala Leu Leu Lys
100 105 110

Ser Lys Leu Arg Ala Leu Leu Thr Ala Pro Arg Ser Leu Arg Arg Ser
115 120 125

Ser Cys Phe Gly Gly Arg Met Asp Arg Ile Gly Ala Gln Ser Gly Leu
130 135 140

Gly Cys Asn Ser Phe Arg Tyr
145 150

<210> 404
<211> 151
<212> PRT
<213> Homo sapiens

<400> 404
Met Ser Ser Phe Ser Thr Thr Thr Val Ser Phe Leu Leu Leu Leu Ala
1 5 10 15

Phe Gln Leu Leu Gly Gln Thr Arg Ala Asn Pro Met Tyr Asn Ala Val
20 25 30

Ser Asn Ala Asp Leu Met Asp Phe Lys Asn Leu Leu Asp His Leu Glu
35 40 45

Glu Lys Met Pro Leu Glu Asp Glu Val Val Pro Pro Gln Val Leu Ser
50 55 60

Glu Pro Asn Glu Glu Ala Gly Ala Ala Leu Ser Pro Leu Pro Glu Val
65 70 75 80

Pro Pro Trp Thr Gly Glu Val Ser Pro Ala Gln Arg Asp Gly Gly Ala
85 90 95

Leu Gly Arg Gly Pro Trp Asp Ser Ser Asp Arg Ser Ala Leu Leu Lys
100 105 110

Ser Lys Leu Arg Ala Leu Leu Thr Ala Pro Arg Ser Leu Arg Arg Ser
115 120 125

Ser Cys Phe Gly Gly Arg Met Asp Arg Ile Gly Ala Gln Ser Gly Leu
130 135 140

Gly Cys Asn Ser Phe Arg Tyr
145 150

<210> 405
<211> 151
<212> PRT
<213> Homo sapiens

<400> 405
Met Ser Ser Phe Ser Thr Thr Thr Val Ser Phe Leu Leu Leu Leu Ala
1 5 10 15

Phe Gln Leu Leu Gly Gln Thr Arg Ala Asn Pro Met Tyr Asn Ala Val
20 25 30

Ser Asn Ala Asp Leu Met Asp Phe Lys Asn Leu Leu Asp His Leu Glu
35 40 45

Glu Lys Met Pro Leu Glu Asp Glu Val Val Pro Pro Gln Val Leu Ser
50 55 60

Glu Pro Asn Glu Glu Ala Gly Ala Ala Leu Ser Pro Leu Pro Glu Val
65 70 75 80

Pro Pro Trp Thr Gly Glu Val Ser Pro Ala Gln Arg Asp Gly Gly Ala
85 90 95

Leu Gly Arg Gly Pro Trp Asp Ser Ser Asp Arg Ser Ala Leu Leu Lys
100 105 110

Ser Lys Leu Arg Ala Leu Leu Thr Ala Pro Arg Ser Leu Arg Arg Ser
115 120 125

Ser Cys Phe Gly Gly Arg Met Asp Arg Ile Gly Ala Gln Ser Gly Leu
130 135 140

Gly Cys Asn Ser Phe Arg Tyr
145 150

<210> 406
<211> 151
<212> PRT
<213> Homo sapiens

<400> 406
Met Ser Ser Phe Ser Thr Thr Thr Val Ser Phe Leu Leu Leu Leu Ala
1 5 10 15

Phe Gln Leu Leu Gly Gln Thr Arg Ala Asn Pro Met Tyr Asn Ala Val
20 25 30

Ser Asn Ala Asp Leu Met Asp Phe Lys Asn Leu Leu Asp His Leu Glu
35 40 45

Glu Lys Met Pro Leu Glu Asp Glu Val Val Pro Pro Gln Val Leu Ser
50 55 60

Glu Pro Asn Glu Glu Ala Gly Ala Ala Leu Ser Pro Leu Pro Glu Val
65 70 75 80

Pro Pro Trp Thr Gly Glu Val Ser Pro Ala Gln Arg Asp Gly Gly Ala

85 90 95

Leu Gly Arg Gly Pro Trp Asp Ser Ser Asp Arg Ser Ala Leu Leu Lys
100 105 110

Ser Lys Leu Arg Ala Leu Leu Thr Ala Pro Arg Ser Leu Arg Arg Ser
115 120 125

Ser Cys Phe Gly Gly Arg Met Asp Arg Ile Gly Ala Gln Ser Gly Leu
130 135 140

Gly Cys Asn Ser Phe Arg Tyr
145 150

<210> 407
<211> 151
<212> PRT
<213> Homo sapiens

<400> 407
Met Ser Ser Phe Ser Thr Thr Thr Val Ser Phe Leu Leu Leu Leu Ala
1 5 10 15

Phe Gln Leu Leu Gly Gln Thr Arg Ala Asn Pro Met Tyr Asn Ala Val
20 25 30

Ser Asn Ala Asp Leu Met Asp Phe Lys Asn Leu Leu Asp His Leu Glu
35 40 45

Glu Lys Met Pro Leu Glu Asp Glu Val Val Pro Pro Gln Val Leu Ser
50 55 60

Glu Pro Asn Glu Glu Ala Gly Ala Ala Leu Ser Pro Leu Pro Glu Val
65 70 75 80

Pro Pro Trp Thr Gly Glu Val Ser Pro Ala Gln Arg Asp Gly Gly Ala
85 90 95

Leu Gly Arg Gly Pro Trp Asp Ser Ser Asp Arg Ser Ala Leu Leu Lys
100 105 110

Ser Lys Leu Arg Ala Leu Leu Thr Ala Pro Arg Ser Leu Arg Arg Ser
115 120 125

Ser Cys Phe Gly Gly Arg Met Asp Arg Ile Gly Ala Gln Ser Gly Leu
130 135 140

Gly Cys Asn Ser Phe Arg Tyr
145 150

<210> 408
<211> 126
<212> PRT
<213> Homo sapiens

<400> 408
Met His Leu Ser Gln Leu Leu Ala Cys Ala Leu Leu Leu Thr Leu Leu

1 5 10 15

Ser Leu Arg Pro Ser Glu Ala Lys Pro Gly Ala Pro Pro Lys Val Pro
20 25 30

Arg Thr Pro Pro Ala Glu Glu Leu Ala Glu Pro Gln Ala Ala Gly Gly
35 40 45

Gly Gln Lys Lys Gly Asp Lys Ala Pro Gly Gly Gly Gly Ala Asn Leu
50 55 60

Lys Gly Asp Arg Ser Arg Leu Leu Arg Asp Leu Arg Val Asp Thr Lys
65 70 75 80

Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg
85 90 95

Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly
100 105 110

Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
115 120 125

<210> 409
<211> 126
<212> PRT
<213> Homo sapiens

<400> 409
Met His Leu Ser Gln Leu Leu Ala Cys Ala Leu Leu Leu Thr Leu Leu
1 5 10 15

Ser Leu Arg Pro Ser Glu Ala Lys Pro Gly Ala Pro Pro Lys Val Pro
20 25 30

Arg Thr Pro Pro Ala Glu Glu Leu Ala Glu Pro Gln Ala Ala Gly Gly
35 40 45

Gly Gln Lys Lys Gly Asp Lys Ala Pro Gly Gly Gly Gly Ala Asn Leu
50 55 60

Lys Gly Asp Arg Ser Arg Leu Leu Arg Asp Leu Arg Val Asp Thr Lys
65 70 75 80

Ser Arg Ala Ala Trp Ala Arg Leu Leu Gln Glu His Pro Asn Ala Arg
85 90 95

Lys Tyr Lys Gly Ala Asn Lys Lys Gly Leu Ser Lys Gly Cys Phe Gly
100 105 110

Leu Lys Leu Asp Arg Ile Gly Ser Met Ser Gly Leu Gly Cys
115 120 125

<210> 410
<211> 38
<212> PRT
<213> Dendroaspis angusticeps

<400> 410
Glu Val Lys Tyr Asp Pro Cys Phe Gly His Lys Ile Asp Arg Ile Asn
1 5 10 15

His Val Ser Asn Leu Gly Cys Pro Ser Leu Arg Asp Pro Arg Pro Asn
20 25 30

Ala Pro Ser Thr Ser Ala
35

<210> 411
<211> 38
<212> PRT
<213> Dendroaspis angusticeps

<400> 411
Glu Val Lys Tyr Asp Pro Cys Phe Gly His Lys Ile Asp Arg Ile Asn
1 5 10 15

His Val Ser Asn Leu Gly Cys Pro Ser Leu Arg Asp Pro Arg Pro Asn
20 25 30

Ala Pro Ser Thr Ser Ala
35

<210> 412
<211> 30
<212> PRT
<213> Homo sapiens

<400> 412
Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met Asp
1 5 10 15

Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg
20 25 30

<210> 413
<211> 32
<212> PRT
<213> Homo sapiens

<400> 413
Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Gly Met Asp
1 5 10 15

Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His
20 25 30

<210> 414
<211> 32
<212> PRT
<213> Homo sapiens

<400> 414

Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Gly Met Asp
1 5 10 15

Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His
20 25 30

<210> 415
<211> 32
<212> PRT
<213> Homo sapiens

<400> 415
Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Gly Met Asp
1 5 10 15

Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His
20 25 30

<210> 416
<211> 32
<212> PRT
<213> Homo sapiens

<400> 416
Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Gly Met Asp
1 5 10 15

Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His
20 25 30

<210> 417
<211> 303
<212> PRT
<213> Rattus norvegicus

<400> 417
Met Ala His Tyr His Asp Asp Tyr Gly Lys Asn Asp Glu Val Glu Phe
1 5 10 15

Val Arg Thr Gly Tyr Gly Lys Asp Met Val Lys Val Leu His Ile Gln
20 25 30

Arg Asp Gly Lys Tyr His Ser Ile Lys Glu Val Ala Thr Ser Val Gln
35 40 45

Leu Thr Leu Arg Ser Lys Lys Asp Tyr Leu His Gly Asp Asn Ser Asp
50 55 60

Ile Ile Pro Thr Asp Thr Ile Lys Asn Thr Val His Val Leu Ala Lys
65 70 75 80

Phe Lys Gly Ile Lys Ser Ile Glu Thr Phe Ala Met Asn Ile Cys Glu
85 90 95

His Phe Leu Ser Ser Phe Ser His Val Thr Arg Ala His Val Tyr Val
100 105 110

Glu Glu Val Pro Trp Lys Arg Phe Glu Lys Asn Gly Val Lys His Val

115 120 125

His Ala Phe Ile His Thr Pro Thr Gly Thr His Phe Cys Asp Val Glu
130 135 140

Gln Val Arg Asn Gly Pro Pro Ile Ile His Ser Gly Ile Lys Asp Leu
145 150 155 160

Lys Val Leu Lys Thr Thr Gln Ser Gly Phe Glu Gly Phe Ile Lys Asp
165 170 175

Gln Phe Thr Thr Leu Pro Glu Val Lys Asp Arg Cys Phe Ala Thr Gln
180 185 190

Val Tyr Cys Lys Trp Arg Tyr Gln Asn Arg Asp Val Asp Phe Glu Ala
195 200 205

Thr Trp Gly Ala Val Arg Asp Ile Val Leu Lys Lys Phe Ala Gly Pro
210 215 220

Tyr Asp Arg Gly Glu Tyr Ser Pro Ser Val Gln Lys Thr Leu Tyr Asp
225 230 235 240

Ile Gln Val Leu Thr Leu Ser Gln Leu Pro Glu Ile Glu Asp Met Glu
245 250 255

Ile Ser Leu Pro Asn Ile His Tyr Phe Asn Ile Asp Met Ser Lys Met
260 265 270

Gly Leu Ile Asn Lys Glu Glu Val Leu Leu Pro Leu Asp Asn Pro Tyr
275 280 285

Gly Lys Ile Thr Gly Thr Val Arg Arg Lys Leu Pro Ser Arg Leu
290 295 300

<210> 418
<211> 303
<212> PRT
<213> Rattus norvegicus

<400> 418
Met Ala His Tyr His Asp Asp Tyr Gly Lys Asn Asp Glu Val Glu Phe
1 5 10 15

Val Arg Thr Gly Tyr Gly Lys Asp Met Val Lys Val Leu His Ile Gln
20 25 30

Arg Asp Gly Lys Tyr His Ser Ile Lys Glu Val Ala Thr Ser Val Gln
35 40 45

Leu Thr Leu Arg Ser Lys Lys Asp Tyr Leu His Gly Asp Asn Ser Asp
50 55 60

Ile Ile Pro Thr Asp Thr Ile Lys Asn Thr Val His Val Leu Ala Lys
65 70 75 80

Phe Lys Gly Ile Lys Ser Ile Glu Thr Phe Ala Met Asn Ile Cys Glu
85 90 95

His Phe Leu Ser Ser Phe Ser His Val Thr Arg Ala His Val Tyr Val
100 105 110

Glu Glu Val Pro Trp Lys Arg Phe Glu Lys Asn Gly Val Lys His Val
115 120 125

His Ala Phe Ile His Thr Pro Thr Gly Thr His Phe Cys Asp Val Glu
130 135 140

Gln Val Arg Asn Gly Pro Pro Ile Ile His Ser Gly Ile Lys Asp Leu
145 150 155 160

Lys Val Leu Lys Thr Thr Gln Ser Gly Phe Glu Gly Phe Ile Lys Asp
165 170 175

Gln Phe Thr Thr Leu Pro Glu Val Lys Asp Arg Cys Phe Ala Thr Gln
180 185 190

Val Tyr Cys Lys Trp Arg Tyr Gln Asn Arg Asp Val Asp Phe Glu Ala
195 200 205

Thr Trp Gly Ala Val Arg Asp Ile Val Leu Lys Lys Phe Ala Gly Pro
210 215 220

Tyr Asp Arg Gly Glu Tyr Ser Pro Ser Val Gln Lys Thr Leu Tyr Asp
225 230 235 240

Ile Gln Val Leu Thr Leu Ser Gln Leu Pro Glu Ile Glu Asp Met Glu
245 250 255

Ile Ser Leu Pro Asn Ile His Tyr Phe Asn Ile Asp Met Ser Lys Met
260 265 270

Gly Leu Ile Asn Lys Glu Glu Val Leu Leu Pro Leu Asp Asn Pro Tyr
275 280 285

Gly Lys Ile Thr Gly Thr Val Arg Arg Lys Leu Pro Ser Arg Leu
290 295 300

<210> 419
<211> 410
<212> PRT
<213> Mycoplasma arginini

<400> 419
Met Ser Val Phe Asp Ser Lys Phe Lys Gly Ile His Val Tyr Ser Glu
1 5 10 15

Ile Gly Glu Leu Glu Ser Val Leu Val His Glu Pro Gly Arg Glu Ile
20 25 30

Asp Tyr Ile Thr Pro Ala Arg Leu Asp Glu Leu Leu Phe Ser Ala Ile
35 40 45

Leu Glu Ser His Asp Ala Arg Lys Glu His Lys Gln Phe Val Ala Glu
50 55 60

Leu Lys Ala Asn Asp Ile Asn Val Val Glu Leu Ile Asp Leu Val Ala
65 70 75 80

Glu Thr Tyr Asp Leu Ala Ser Gln Glu Ala Lys Asp Lys Leu Ile Glu
85 90 95

Glu Phe Leu Glu Asp Ser Glu Pro Val Leu Ser Glu Glu His Lys Val
100 105 110

Val Val Arg Asn Phe Leu Lys Ala Lys Lys Thr Ser Arg Lys Leu Val
115 120 125

Glu Ile Met Met Ala Gly Ile Thr Lys Tyr Asp Leu Gly Ile Glu Ala
130 135 140

Asp His Glu Leu Ile Val Asp Pro Met Pro Asn Leu Tyr Phe Thr Arg
145 150 155 160

Asp Pro Phe Ala Ser Val Gly Asn Gly Val Thr Ile His Tyr Met Arg
165 170 175

Tyr Lys Val Arg Gln Arg Glu Thr Leu Phe Ser Arg Phe Val Phe Ser
180 185 190

Asn His Pro Lys Leu Ile Asn Thr Pro Trp Tyr Tyr Asp Pro Ser Leu
195 200 205

Lys Leu Ser Ile Glu Gly Gly Asp Val Phe Ile Tyr Asn Asn Asp Thr
210 215 220

Leu Val Val Gly Val Ser Glu Arg Thr Asp Leu Gln Thr Val Thr Leu
225 230 235 240

Leu Ala Lys Asn Ile Val Ala Asn Lys Glu Cys Glu Phe Lys Arg Ile
245 250 255

Val Ala Ile Asn Val Pro Lys Trp Thr Asn Leu Met His Leu Asp Thr
260 265 270

Trp Leu Thr Met Leu Asp Lys Asp Lys Phe Leu Tyr Ser Pro Ile Ala
275 280 285

Asn Asp Val Phe Lys Phe Trp Asp Tyr Asp Leu Val Asn Gly Gly Ala
290 295 300

Glu Pro Gln Pro Val Glu Asn Gly Leu Pro Leu Glu Gly Leu Leu Gln
305 310 315 320

Ser Ile Ile Asn Lys Lys Pro Val Leu Ile Pro Ile Ala Gly Glu Gly
325 330 335

Ala Ser Gln Met Glu Ile Glu Arg Glu Thr His Phe Asp Gly Thr Asn
340 345 350

Tyr Leu Ala Ile Arg Pro Gly Val Val Ile Gly Tyr Ser Arg Asn Glu
355 360 365

Lys Thr Asn Ala Ala Leu Glu Ala Ala Gly Ile Lys Val Leu Pro Phe
370 375 380

His Gly Asn Gln Leu Ser Leu Gly Met Gly Asn Ala Arg Cys Met Ser
385 390 395 400

Met Pro Leu Ser Arg Lys Asp Val Lys Trp

405 410

<210> 420
<211> 410
<212> PRT
<213> Mycoplasma arginini

<400> 420
Met Ser Val Phe Asp Ser Lys Phe Lys Gly Ile His Val Tyr Ser Glu
1 5 10 15

Ile Gly Glu Leu Glu Ser Val Leu Val His Glu Pro Gly Arg Glu Ile
20 25 30

Asp Tyr Ile Thr Pro Ala Arg Leu Asp Glu Leu Leu Phe Ser Ala Ile
35 40 45

Leu Glu Ser His Asp Ala Arg Lys Glu His Lys Gln Phe Val Ala Glu
50 55 60

Leu Lys Ala Asn Asp Ile Asn Val Val Glu Leu Ile Asp Leu Val Ala
65 70 75 80

Glu Thr Tyr Asp Leu Ala Ser Gln Glu Ala Lys Asp Lys Leu Ile Glu
85 90 95

Glu Phe Leu Glu Asp Ser Glu Pro Val Leu Ser Glu Glu His Lys Val
100 105 110

Val Val Arg Asn Phe Leu Lys Ala Lys Lys Thr Ser Arg Lys Leu Val
115 120 125

Glu Ile Met Met Ala Gly Ile Thr Lys Tyr Asp Leu Gly Ile Glu Ala
130 135 140

Asp His Glu Leu Ile Val Asp Pro Met Pro Asn Leu Tyr Phe Thr Arg
145 150 155 160

Asp Pro Phe Ala Ser Val Gly Asn Gly Val Thr Ile His Tyr Met Arg
165 170 175

Tyr Lys Val Arg Gln Arg Glu Thr Leu Phe Ser Arg Phe Val Phe Ser
180 185 190

Asn His Pro Lys Leu Ile Asn Thr Pro Trp Tyr Tyr Asp Pro Ser Leu
195 200 205

Lys Leu Ser Ile Glu Gly Gly Asp Val Phe Ile Tyr Asn Asn Asp Thr
210 215 220

Leu Val Val Gly Val Ser Glu Arg Thr Asp Leu Gln Thr Val Thr Leu
225 230 235 240

Leu Ala Lys Asn Ile Val Ala Asn Lys Glu Cys Glu Phe Lys Arg Ile
245 250 255

Val Ala Ile Asn Val Pro Lys Trp Thr Asn Leu Met His Leu Asp Thr
260 265 270

Trp Leu Thr Met Leu Asp Lys Asp Lys Phe Leu Tyr Ser Pro Ile Ala
275 280 285

Asn Asp Val Phe Lys Phe Trp Asp Tyr Asp Leu Val Asn Gly Gly Ala
290 295 300

Glu Pro Gln Pro Val Glu Asn Gly Leu Pro Leu Glu Gly Leu Leu Gln
305 310 315 320

Ser Ile Ile Asn Lys Lys Pro Val Leu Ile Pro Ile Ala Gly Glu Gly
325 330 335

Ala Ser Gln Met Glu Ile Glu Arg Glu Thr His Phe Asp Gly Thr Asn
340 345 350

Tyr Leu Ala Ile Arg Pro Gly Val Val Ile Gly Tyr Ser Arg Asn Glu
355 360 365

Lys Thr Asn Ala Ala Leu Glu Ala Ala Gly Ile Lys Val Leu Pro Phe
370 375 380

His Gly Asn Gln Leu Ser Leu Gly Met Gly Asn Ala Arg Cys Met Ser
385 390 395 400

Met Pro Leu Ser Arg Lys Asp Val Lys Trp
405 410

<210> 421
<211> 410
<212> PRT
<213> Mycoplasma arginini

<400> 421
Met Ser Val Phe Asp Ser Lys Phe Lys Gly Ile His Val Tyr Ser Glu
1 5 10 15

Ile Gly Glu Leu Glu Ser Val Leu Val His Glu Pro Gly Arg Glu Ile
20 25 30

Asp Tyr Ile Thr Pro Ala Arg Leu Asp Glu Leu Leu Phe Ser Ala Ile
35 40 45

Leu Glu Ser His Asp Ala Arg Lys Glu His Lys Gln Phe Val Ala Glu
50 55 60

Leu Lys Ala Asn Asp Ile Asn Val Val Glu Leu Ile Asp Leu Val Ala
65 70 75 80

Glu Thr Tyr Asp Leu Ala Ser Gln Glu Ala Lys Asp Lys Leu Ile Glu
85 90 95

Glu Phe Leu Glu Asp Ser Glu Pro Val Leu Ser Glu Glu His Lys Val
100 105 110

Val Val Arg Asn Phe Leu Lys Ala Lys Lys Thr Ser Arg Lys Leu Val
115 120 125

Glu Ile Met Met Ala Gly Ile Thr Lys Tyr Asp Leu Gly Ile Glu Ala
130 135 140

Asp His Glu Leu Ile Val Asp Pro Met Pro Asn Leu Tyr Phe Thr Arg
145 150 155 160

Asp Pro Phe Ala Ser Val Gly Asn Gly Val Thr Ile His Tyr Met Arg
165 170 175

Tyr Lys Val Arg Gln Arg Glu Thr Leu Phe Ser Arg Phe Val Phe Ser
180 185 190

Asn His Pro Lys Leu Ile Asn Thr Pro Trp Tyr Tyr Asp Pro Ser Leu
195 200 205

Lys Leu Ser Ile Glu Gly Gly Asp Val Phe Ile Tyr Asn Asn Asp Thr
210 215 220

Leu Val Val Gly Val Ser Glu Arg Thr Asp Leu Gln Thr Val Thr Leu
225 230 235 240

Leu Ala Lys Asn Ile Val Ala Asn Lys Glu Cys Glu Phe Lys Arg Ile
245 250 255

Val Ala Ile Asn Val Pro Lys Trp Thr Asn Leu Met His Leu Asp Thr
260 265 270

Trp Leu Thr Met Leu Asp Lys Asp Lys Phe Leu Tyr Ser Pro Ile Ala
275 280 285

Asn Asp Val Phe Lys Phe Trp Asp Tyr Asp Leu Val Asn Gly Gly Ala
290 295 300

Glu Pro Gln Pro Val Glu Asn Gly Leu Pro Leu Glu Gly Leu Leu Gln
305 310 315 320

Ser Ile Ile Asn Lys Lys Pro Val Leu Ile Pro Ile Ala Gly Glu Gly
325 330 335

Ala Ser Gln Met Glu Ile Glu Arg Glu Thr His Phe Asp Gly Thr Asn
340 345 350

Tyr Leu Ala Ile Arg Pro Gly Val Val Ile Gly Tyr Ser Arg Asn Glu
355 360 365

Lys Thr Asn Ala Ala Leu Glu Ala Ala Gly Ile Lys Val Leu Pro Phe
370 375 380

His Gly Asn Gln Leu Ser Leu Gly Met Gly Asn Ala Arg Cys Met Ser
385 390 395 400

Met Pro Leu Ser Arg Lys Asp Val Lys Trp
405 410

<210> 422
<211> 410
<212> PRT
<213> Mycoplasma arginini

<400> 422
Met Ser Val Phe Asp Ser Lys Phe Lys Gly Ile His Val Tyr Ser Glu

1 5 10 15

Ile Gly Glu Leu Glu Ser Val Leu Val His Glu Pro Gly Arg Glu Ile
20 25 30

Asp Tyr Ile Thr Pro Ala Arg Leu Asp Glu Leu Leu Phe Ser Ala Ile
35 40 45

Leu Glu Ser His Asp Ala Arg Lys Glu His Lys Gln Phe Val Ala Glu
50 55 60

Leu Lys Ala Asn Asp Ile Asn Val Val Glu Leu Ile Asp Leu Val Ala
65 70 75 80

Glu Thr Tyr Asp Leu Ala Ser Gln Glu Ala Lys Asp Lys Leu Ile Glu
85 90 95

Glu Phe Leu Glu Asp Ser Glu Pro Val Leu Ser Glu Glu His Lys Val
100 105 110

Val Val Arg Asn Phe Leu Lys Ala Lys Lys Thr Ser Arg Lys Leu Val
115 120 125

Glu Ile Met Met Ala Gly Ile Thr Lys Tyr Asp Leu Gly Ile Glu Ala
130 135 140

Asp His Glu Leu Ile Val Asp Pro Met Pro Asn Leu Tyr Phe Thr Arg
145 150 155 160

Asp Pro Phe Ala Ser Val Gly Asn Gly Val Thr Ile His Tyr Met Arg
165 170 175

Tyr Lys Val Arg Gln Arg Glu Thr Leu Phe Ser Arg Phe Val Phe Ser
180 185 190

Asn His Pro Lys Leu Ile Asn Thr Pro Trp Tyr Tyr Asp Pro Ser Leu
195 200 205

Lys Leu Ser Ile Glu Gly Gly Asp Val Phe Ile Tyr Asn Asn Asp Thr
210 215 220

Leu Val Val Gly Val Ser Glu Arg Thr Asp Leu Gln Thr Val Thr Leu
225 230 235 240

Leu Ala Lys Asn Ile Val Ala Asn Lys Glu Cys Glu Phe Lys Arg Ile
245 250 255

Val Ala Ile Asn Val Pro Lys Trp Thr Asn Leu Met His Leu Asp Thr
260 265 270

Trp Leu Thr Met Leu Asp Lys Asp Lys Phe Leu Tyr Ser Pro Ile Ala
275 280 285

Asn Asp Val Phe Lys Phe Trp Asp Tyr Asp Leu Val Asn Gly Gly Ala
290 295 300

Glu Pro Gln Pro Val Glu Asn Gly Leu Pro Leu Glu Gly Leu Leu Gln
305 310 315 320

Ser Ile Ile Asn Lys Lys Pro Val Leu Ile Pro Ile Ala Gly Glu Gly
325 330 335

Ala Ser Gln Met Glu Ile Glu Arg Glu Thr His Phe Asp Gly Thr Asn
340 345 350

Tyr Leu Ala Ile Arg Pro Gly Val Val Ile Gly Tyr Ser Arg Asn Glu
355 360 365

Lys Thr Asn Ala Ala Leu Glu Ala Ala Gly Ile Lys Val Leu Pro Phe
370 375 380

His Gly Asn Gln Leu Ser Leu Gly Met Gly Asn Ala Arg Cys Met Ser
385 390 395 400

Met Pro Leu Ser Arg Lys Asp Val Lys Trp
405 410

<210> 423
<211> 129
<212> PRT
<213> Homo sapiens

<400> 423
Met Ala Arg Gly Ser Leu Arg Arg Leu Leu Arg Leu Leu Val Leu Gly
1 5 10 15

Leu Trp Leu Ala Leu Leu Arg Ser Val Ala Gly Glu Gln Ala Pro Gly
20 25 30

Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
35 40 45

Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
50 55 60

Leu Gly Cys Ala Ala Ala Pro Pro Ala Pro Phe Arg Leu Leu Trp Pro
65 70 75 80

Ile Leu Gly Gly Ala Leu Ser Leu Thr Phe Val Leu Gly Leu Leu Ser
85 90 95

Gly Phe Leu Val Trp Arg Arg Cys Arg Arg Arg Glu Lys Phe Thr Thr
100 105 110

Pro Ile Glu Glu Thr Gly Gly Glu Gly Cys Pro Ala Val Ala Leu Ile
115 120 125

Gln

<210> 424
<211> 497
<212> PRT
<213> Homo sapiens

<400> 424
Ala Arg Pro Cys Ile Pro Lys Ser Phe Gly Tyr Ser Ser Val Val Cys
1 5 10 15

Val Cys Asn Ala Thr Tyr Cys Asp Ser Phe Asp Pro Pro Thr Phe Pro

20 25 30

Ala Leu Gly Thr Phe Ser Arg Tyr Glu Ser Thr Arg Ser Gly Arg Arg
35 40 45

Met Glu Leu Ser Met Gly Pro Ile Gln Ala Asn His Thr Gly Thr Gly
50 55 60

Leu Leu Leu Thr Leu Gln Pro Glu Gln Lys Phe Gln Lys Val Lys Gly
65 70 75 80

Phe Gly Gly Ala Met Thr Asp Ala Ala Ala Leu Asn Ile Leu Ala Leu
85 90 95

Ser Pro Pro Ala Gln Asn Leu Leu Leu Lys Ser Tyr Phe Ser Glu Glu
100 105 110

Gly Ile Gly Tyr Asn Ile Ile Arg Val Pro Met Ala Ser Cys Asp Phe
115 120 125

Ser Ile Arg Thr Tyr Thr Tyr Ala Asp Thr Pro Asp Asp Phe Gln Leu
130 135 140

His Asn Phe Ser Leu Pro Glu Glu Asp Thr Lys Leu Lys Ile Pro Leu
145 150 155 160

Ile His Arg Ala Leu Gln Leu Ala Gln Arg Pro Val Ser Leu Leu Ala
165 170 175

Ser Pro Trp Thr Ser Pro Thr Trp Leu Lys Thr Asn Gly Ala Val Asn
180 185 190

Gly Lys Gly Ser Leu Lys Gly Gln Pro Gly Asp Ile Tyr His Gln Thr
195 200 205

Trp Ala Arg Tyr Phe Val Lys Phe Leu Asp Ala Tyr Ala Glu His Lys
210 215 220

Leu Gln Phe Trp Ala Val Thr Ala Glu Asn Glu Pro Ser Ala Gly Leu
225 230 235 240

Leu Ser Gly Tyr Pro Phe Gln Cys Leu Gly Phe Thr Pro Glu His Gln
245 250 255

Arg Asp Phe Ile Ala Arg Asp Leu Gly Pro Thr Leu Ala Asn Ser Thr
260 265 270

His His Asn Val Arg Leu Leu Met Leu Asp Asp Gln Arg Leu Leu Leu
275 280 285

Pro His Trp Ala Lys Val Val Leu Thr Asp Pro Glu Ala Ala Lys Tyr
290 295 300

Val His Gly Ile Ala Val His Trp Tyr Leu Asp Phe Leu Ala Pro Ala
305 310 315 320

Lys Ala Thr Leu Gly Glu Thr His Arg Leu Phe Pro Asn Thr Met Leu
325 330 335

Phe Ala Ser Glu Ala Cys Val Gly Ser Lys Phe Trp Glu Gln Ser Val
340 345 350

Arg Leu Gly Ser Trp Asp Arg Gly Met Gln Tyr Ser His Ser Ile Ile
355 360 365

Thr Asn Leu Leu Tyr His Val Val Gly Trp Thr Asp Trp Asn Leu Ala
370 375 380

Leu Asn Pro Glu Gly Gly Pro Asn Trp Val Arg Asn Phe Val Asp Ser
385 390 395 400

Pro Ile Ile Val Asp Ile Thr Lys Asp Thr Phe Tyr Lys Gln Pro Met
405 410 415

Phe Tyr His Leu Gly His Phe Ser Lys Phe Ile Pro Glu Gly Ser Gln
420 425 430

Arg Val Gly Leu Val Ala Ser Gln Lys Asn Asp Leu Asp Ala Val Ala
435 440 445

Leu Met His Pro Asp Gly Ser Ala Val Val Val Val Leu Asn Arg Ser
450 455 460

Ser Lys Asp Val Pro Leu Thr Ile Lys Asp Pro Ala Val Gly Phe Leu
465 470 475 480

Glu Thr Ile Ser Pro Gly Tyr Ser Ile His Thr Tyr Leu Trp Arg Arg
485 490 495

Gln

<210> 425
<211> 497
<212> PRT
<213> Homo sapiens

<400> 425
Ala Arg Pro Cys Ile Pro Lys Ser Phe Gly Tyr Ser Ser Val Val Cys
1 5 10 15

Val Cys Asn Ala Thr Tyr Cys Asp Ser Phe Asp Pro Pro Thr Phe Pro
20 25 30

Ala Leu Gly Thr Phe Ser Arg Tyr Glu Ser Thr Arg Ser Gly Arg Arg
35 40 45

Met Glu Leu Ser Met Gly Pro Ile Gln Ala Asn His Thr Gly Thr Gly
50 55 60

Leu Leu Leu Thr Leu Gln Pro Glu Gln Lys Phe Gln Lys Val Lys Gly
65 70 75 80

Phe Gly Gly Ala Met Thr Asp Ala Ala Ala Leu Asn Ile Leu Ala Leu
85 90 95

Ser Pro Pro Ala Gln Asn Leu Leu Leu Lys Ser Tyr Phe Ser Glu Glu
100 105 110

Gly Ile Gly Tyr Asn Ile Ile Arg Val Pro Met Ala Ser Cys Asp Phe
115 120 125

Ser Ile Arg Thr Tyr Thr Tyr Ala Asp Thr Pro Asp Asp Phe Gln Leu
130 135 140

His Asn Phe Ser Leu Pro Glu Glu Asp Thr Lys Leu Lys Ile Pro Leu
145 150 155 160

Ile His Arg Ala Leu Gln Leu Ala Gln Arg Pro Val Ser Leu Leu Ala
165 170 175

Ser Pro Trp Thr Ser Pro Thr Trp Leu Lys Thr Asn Gly Ala Val Asn
180 185 190

Gly Lys Gly Ser Leu Lys Gly Gln Pro Gly Asp Ile Tyr His Gln Thr
195 200 205

Trp Ala Arg Tyr Phe Val Lys Phe Leu Asp Ala Tyr Ala Glu His Lys
210 215 220

Leu Gln Phe Trp Ala Val Thr Ala Glu Asn Glu Pro Ser Ala Gly Leu
225 230 235 240

Leu Ser Gly Tyr Pro Phe Gln Cys Leu Gly Phe Thr Pro Glu His Gln
245 250 255

Arg Asp Phe Ile Ala Arg Asp Leu Gly Pro Thr Leu Ala Asn Ser Thr
260 265 270

His His Asn Val Arg Leu Leu Met Leu Asp Asp Gln Arg Leu Leu Leu
275 280 285

Pro His Trp Ala Lys Val Val Leu Thr Asp Pro Glu Ala Ala Lys Tyr
290 295 300

Val His Gly Ile Ala Val His Trp Tyr Leu Asp Phe Leu Ala Pro Ala
305 310 315 320

Lys Ala Thr Leu Gly Glu Thr His Arg Leu Phe Pro Asn Thr Met Leu
325 330 335

Phe Ala Ser Glu Ala Cys Val Gly Ser Lys Phe Trp Glu Gln Ser Val
340 345 350

Arg Leu Gly Ser Trp Asp Arg Gly Met Gln Tyr Ser His Ser Ile Ile
355 360 365

Thr Asn Leu Leu Tyr His Val Val Gly Trp Thr Asp Trp Asn Leu Ala
370 375 380

Leu Asn Pro Glu Gly Gly Pro Asn Trp Val Arg Asn Phe Val Asp Ser
385 390 395 400

Pro Ile Ile Val Asp Ile Thr Lys Asp Thr Phe Tyr Lys Gln Pro Met
405 410 415

Phe Tyr His Leu Gly His Phe Ser Lys Phe Ile Pro Glu Gly Ser Gln
420 425 430

Arg Val Gly Leu Val Ala Ser Gln Lys Asn Asp Leu Asp Ala Val Ala
435 440 445

Leu Met His Pro Asp Gly Ser Ala Val Val Val Val Leu Asn Arg Ser

450 455 460

Ser Lys Asp Val Pro Leu Thr Ile Lys Asp Pro Ala Val Gly Phe Leu
465 470 475 480

Glu Thr Ile Ser Pro Gly Tyr Ser Ile His Thr Tyr Leu Trp Arg Arg
485 490 495

Gln

<210> 426
<211> 497
<212> PRT
<213> Homo sapiens

<400> 426
Ala Arg Pro Cys Ile Pro Lys Ser Phe Gly Tyr Ser Ser Val Val Cys
1 5 10 15

Val Cys Asn Ala Thr Tyr Cys Asp Ser Phe Asp Pro Pro Thr Phe Pro
20 25 30

Ala Leu Gly Thr Phe Ser Arg Tyr Glu Ser Thr Arg Ser Gly Arg Arg
35 40 45

Met Glu Leu Ser Met Gly Pro Ile Gln Ala Asn His Thr Gly Thr Gly
50 55 60

Leu Leu Leu Thr Leu Gln Pro Glu Gln Lys Phe Gln Lys Val Lys Gly
65 70 75 80

Phe Gly Gly Ala Met Thr Asp Ala Ala Ala Leu Asn Ile Leu Ala Leu
85 90 95

Ser Pro Pro Ala Gln Asn Leu Leu Leu Lys Ser Tyr Phe Ser Glu Glu
100 105 110

Gly Ile Gly Tyr Asn Ile Ile Arg Val Pro Met Ala Ser Cys Asp Phe
115 120 125

Ser Ile Arg Thr Tyr Thr Tyr Ala Asp Thr Pro Asp Asp Phe Gln Leu
130 135 140

His Asn Phe Ser Leu Pro Glu Glu Asp Thr Lys Leu Lys Ile Pro Leu
145 150 155 160

Ile His Arg Ala Leu Gln Leu Ala Gln Arg Pro Val Ser Leu Leu Ala
165 170 175

Ser Pro Trp Thr Ser Pro Thr Trp Leu Lys Thr Asn Gly Ala Val Asn
180 185 190

Gly Lys Gly Ser Leu Lys Gly Gln Pro Gly Asp Ile Tyr His Gln Thr
195 200 205

Trp Ala Arg Tyr Phe Val Lys Phe Leu Asp Ala Tyr Ala Glu His Lys
210 215 220

Leu Gln Phe Trp Ala Val Thr Ala Glu Asn Glu Pro Ser Ala Gly Leu

225 230 235 240

Leu Ser Gly Tyr Pro Phe Gln Cys Leu Gly Phe Thr Pro Glu His Gln
245 250 255

Arg Asp Phe Ile Ala Arg Asp Leu Gly Pro Thr Leu Ala Asn Ser Thr
260 265 270

His His Asn Val Arg Leu Leu Met Leu Asp Asp Gln Arg Leu Leu Leu
275 280 285

Pro His Trp Ala Lys Val Val Leu Thr Asp Pro Glu Ala Ala Lys Tyr
290 295 300

Val His Gly Ile Ala Val His Trp Tyr Leu Asp Phe Leu Ala Pro Ala
305 310 315 320

Lys Ala Thr Leu Gly Glu Thr His Arg Leu Phe Pro Asn Thr Met Leu
325 330 335

Phe Ala Ser Glu Ala Cys Val Gly Ser Lys Phe Trp Glu Gln Ser Val
340 345 350

Arg Leu Gly Ser Trp Asp Arg Gly Met Gln Tyr Ser His Ser Ile Ile
355 360 365

Thr Asn Leu Leu Tyr His Val Val Gly Trp Thr Asp Trp Asn Leu Ala
370 375 380

Leu Asn Pro Glu Gly Gly Pro Asn Trp Val Arg Asn Phe Val Asp Ser
385 390 395 400

Pro Ile Ile Val Asp Ile Thr Lys Asp Thr Phe Tyr Lys Gln Pro Met
405 410 415

Phe Tyr His Leu Gly His Phe Ser Lys Phe Ile Pro Glu Gly Ser Gln
420 425 430

Arg Val Gly Leu Val Ala Ser Gln Lys Asn Asp Leu Asp Ala Val Ala
435 440 445

Leu Met His Pro Asp Gly Ser Ala Val Val Val Val Leu Asn Arg Ser
450 455 460

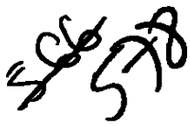
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465 470 475 480

Glu Thr Ile Ser Pro Gly Tyr Ser Ile His Thr Tyr Leu Trp Arg Arg
485 490 495

Gln

<210> 427
<211> 497
<212> PRT
<213> Homo sapiens

<400> 427
Ala Arg Pro Cys Ile Pro Lys Ser Phe Gly Tyr Ser Ser Val Val Cys



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 1               5                  10                  15

Val Cys Asn Ala Thr Tyr Cys Asp Ser Phe Asp Pro Pro Thr Phe Pro
            20                  25                  30

Ala Leu Gly Thr Phe Ser Arg Tyr Glu Ser Thr Arg Ser Gly Arg Arg
        35                  40                  45

Met Glu Leu Ser Met Gly Pro Ile Gln Ala Asn His Thr Gly Thr Gly
    50                  55                  60

Leu Leu Leu Thr Leu Gln Pro Glu Gln Lys Phe Gln Lys Val Lys Gly
65                  70                  75                  80

Phe Gly Gly Ala Met Thr Asp Ala Ala Ala Leu Asn Ile Leu Ala Leu
                85                  90                  95

Ser Pro Pro Ala Gln Asn Leu Leu Leu Lys Ser Tyr Phe Ser Glu Glu
            100                 105                 110

Gly Ile Gly Tyr Asn Ile Ile Arg Val Pro Met Ala Ser Cys Asp Phe
        115                 120                 125

Ser Ile Arg Thr Tyr Thr Tyr Ala Asp Thr Pro Asp Asp Phe Gln Leu
    130                 135                 140

His Asn Phe Ser Leu Pro Glu Glu Asp Thr Lys Leu Lys Ile Pro Leu
145                 150                 155                 160

Ile His Arg Ala Leu Gln Leu Ala Gln Arg Pro Val Ser Leu Leu Ala
                165                 170                 175

Ser Pro Trp Thr Ser Pro Thr Trp Leu Lys Thr Asn Gly Ala Val Asn
            180                 185                 190

Gly Lys Gly Ser Leu Lys Gly Gln Pro Gly Asp Ile Tyr His Gln Thr
        195                 200                 205

Trp Ala Arg Tyr Phe Val Lys Phe Leu Asp Ala Tyr Ala Glu His Lys
    210                 215                 220

Leu Gln Phe Trp Ala Val Thr Ala Glu Asn Glu Pro Ser Ala Gly Leu
225                 230                 235                 240

Leu Ser Gly Tyr Pro Phe Gln Cys Leu Gly Phe Thr Pro Glu His Gln
                245                 250                 255

Arg Asp Phe Ile Ala Arg Asp Leu Gly Pro Thr Leu Ala Asn Ser Thr
            260                 265                 270

His His Asn Val Arg Leu Leu Met Leu Asp Asp Gln Arg Leu Leu Leu
        275                 280                 285

Pro His Trp Ala Lys Val Val Leu Thr Asp Pro Glu Ala Ala Lys Tyr
    290                 295                 300

Val His Gly Ile Ala Val His Trp Tyr Leu Asp Phe Leu Ala Pro Ala
305                 310                 315                 320

Lys Ala Thr Leu Gly Glu Thr His Arg Leu Phe Pro Asn Thr Met Leu
                325                 330                 335
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Phe Ala Ser Glu Ala Cys Val Gly Ser Lys Phe Trp Glu Gln Ser Val
340 345 350

Arg Leu Gly Ser Trp Asp Arg Gly Met Gln Tyr Ser His Ser Ile Ile
355 360 365

Thr Asn Leu Leu Tyr His Val Val Gly Trp Thr Asp Trp Asn Leu Ala
370 375 380

Leu Asn Pro Glu Gly Gly Pro Asn Trp Val Arg Asn Phe Val Asp Ser
385 390 395 400

Pro Ile Ile Val Asp Ile Thr Lys Asp Thr Phe Tyr Lys Gln Pro Met
405 410 415

Phe Tyr His Leu Gly His Phe Ser Lys Phe Ile Pro Glu Gly Ser Gln
420 425 430

Arg Val Gly Leu Val Ala Ser Gln Lys Asn Asp Leu Asp Ala Val Ala
435 440 445

Leu Met His Pro Asp Gly Ser Ala Val Val Val Val Leu Asn Arg Ser
450 455 460

Ser Lys Asp Val Pro Leu Thr Ile Lys Asp Pro Ala Val Gly Phe Leu
465 470 475 480

Glu Thr Ile Ser Pro Gly Tyr Ser Ile His Thr Tyr Leu Trp Arg Arg
485 490 495

Gln

<210> 428
<211> 52
<212> PRT
<213> Homo sapiens

<400> 428
Tyr Arg Gln Ser Met Asn Asn Phe Gln Gly Leu Arg Ser Phe Gly Cys
1 5 10 15

Arg Phe Gly Thr Cys Thr Val Gln Lys Leu Ala His Gln Ile Tyr Gln
20 25 30

Phe Thr Asp Lys Asp Lys Asp Asn Val Ala Pro Arg Ser Lys Ile Ser
35 40 45

Pro Gln Gly Tyr
50

<210> 429
<211> 52
<212> PRT
<213> Homo sapiens

<400> 429
Tyr Arg Gln Ser Met Asn Asn Phe Gln Gly Leu Arg Ser Phe Gly Cys
1 5 10 15

Arg Phe Gly Thr Cys Thr Val Gln Lys Leu Ala His Gln Ile Tyr Gln
20 25 30

Phe Thr Asp Lys Asp Lys Asp Asn Val Ala Pro Arg Ser Lys Ile Ser
35 40 45

Pro Gln Gly Tyr
50

<210> 430
<211> 52
<212> PRT
<213> Homo sapiens

<400> 430
Tyr Arg Gln Ser Met Asn Asn Phe Gln Gly Leu Arg Ser Phe Gly Cys
1 5 10 15

Arg Phe Gly Thr Cys Thr Val Gln Lys Leu Ala His Gln Ile Tyr Gln
20 25 30

Phe Thr Asp Lys Asp Lys Asp Asn Val Ala Pro Arg Ser Lys Ile Ser
35 40 45

Pro Gln Gly Tyr
50

<210> 431
<211> 52
<212> PRT
<213> Homo sapiens

<400> 431
Tyr Arg Gln Ser Met Asn Asn Phe Gln Gly Leu Arg Ser Phe Gly Cys
1 5 10 15

Arg Phe Gly Thr Cys Thr Val Gln Lys Leu Ala His Gln Ile Tyr Gln
20 25 30

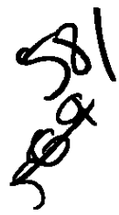
Phe Thr Asp Lys Asp Lys Asp Asn Val Ala Pro Arg Ser Lys Ile Ser
35 40 45

Pro Gln Gly Tyr
50

<210> 432
<211> 145
<212> PRT
<213> Homo sapiens

<400> 432
Met Asn Ser Leu Val Ser Trp Gln Leu Leu Leu Phe Leu Cys Ala Thr
1 5 10 15

His Phe Gly Glu Pro Leu Glu Lys Val Ala Ser Val Gly Asn Ser Arg
20 25 30



Pro Thr Gly Gln Gln Leu Glu Ser Leu Gly Leu Leu Ala Pro Gly Glu
35 40 45

Gln Ser Leu Pro Cys Thr Glu Arg Lys Pro Ala Ala Thr Ala Arg Leu
50 55 60

Ser Arg Arg Gly Thr Ser Leu Ser Pro Pro Pro Glu Ser Ser Gly Ser
65 70 75 80

Pro Gln Gln Pro Gly Leu Ser Ala Pro His Ser Arg Gln Ile Pro Ala
85 90 95

Pro Gln Gly Ala Val Leu Val Gln Arg Glu Lys Asp Leu Pro Asn Tyr
100 105 110

Asn Trp Asn Ser Phe Gly Leu Arg Phe Gly Lys Arg Glu Ala Ala Pro
115 120 125

Gly Asn His Gly Arg Ser Ala Gly Arg Gly Trp Gly Ala Gly Ala Gly
130 135 140

Gln
145

<210> 433
<211> 145
<212> PRT
<213> Homo sapiens

<400> 433
Met Asn Ser Leu Val Ser Trp Gln Leu Leu Leu Phe Leu Cys Ala Thr
1 5 10 15

His Phe Gly Glu Pro Leu Glu Lys Val Ala Ser Val Gly Asn Ser Arg
20 25 30

Pro Thr Gly Gln Gln Leu Glu Ser Leu Gly Leu Leu Ala Pro Gly Glu
35 40 45

Gln Ser Leu Pro Cys Thr Glu Arg Lys Pro Ala Ala Thr Ala Arg Leu
50 55 60

Ser Arg Arg Gly Thr Ser Leu Ser Pro Pro Pro Glu Ser Ser Gly Ser
65 70 75 80

Pro Gln Gln Pro Gly Leu Ser Ala Pro His Ser Arg Gln Ile Pro Ala
85 90 95

Pro Gln Gly Ala Val Leu Val Gln Arg Glu Lys Asp Leu Pro Asn Tyr
100 105 110

Asn Trp Asn Ser Phe Gly Leu Arg Phe Gly Lys Arg Glu Ala Ala Pro
115 120 125

Gly Asn His Gly Arg Ser Ala Gly Arg Gly Trp Gly Ala Gly Ala Gly
130 135 140

Gln
145

582

<210> 434
<211> 346
<212> PRT
<213> Homo sapiens

<400> 434
Met Ala Arg Pro Gly Gln Arg Trp Leu Gly Lys Trp Leu Val Ala Met
1 5 10 15

Val Val Trp Ala Leu Cys Arg Leu Ala Thr Pro Leu Ala Lys Asn Leu
20 25 30

Glu Pro Val Ser Trp Ser Ser Leu Asn Pro Lys Phe Leu Ser Gly Lys
35 40 45

Gly Leu Val Ile Tyr Pro Lys Ile Gly Asp Lys Leu Asp Ile Ile Cys
50 55 60

Pro Arg Ala Glu Ala Gly Arg Pro Tyr Glu Tyr Tyr Lys Leu Tyr Leu
65 70 75 80

Val Arg Pro Glu Gln Ala Ala Ala Cys Ser Thr Val Leu Asp Pro Asn
85 90 95

Val Leu Val Thr Cys Asn Arg Pro Glu Gln Glu Ile Arg Phe Thr Ile
100 105 110

Lys Phe Gln Glu Phe Ser Pro Asn Tyr Met Gly Leu Glu Phe Lys Lys
115 120 125

His His Asp Tyr Tyr Ile Thr Ser Thr Ser Asn Gly Ser Leu Glu Gly
130 135 140

Leu Glu Asn Arg Glu Gly Gly Val Cys Arg Thr Arg Thr Met Lys Ile
145 150 155 160

Ile Met Lys Val Gly Gln Asp Pro Asn Ala Val Thr Pro Glu Gln Leu
165 170 175

Thr Thr Ser Arg Pro Ser Lys Glu Ala Asp Asn Thr Val Lys Met Ala
180 185 190

Thr Gln Ala Pro Gly Ser Arg Gly Ser Leu Gly Asp Ser Asp Gly Lys
195 200 205

His Glu Thr Val Asn Gln Glu Glu Lys Ser Gly Pro Gly Ala Ser Gly
210 215 220

Gly Ser Ser Gly Asp Pro Asp Gly Phe Phe Asn Ser Lys Val Ala Leu
225 230 235 240

Phe Ala Ala Val Gly Ala Gly Cys Val Ile Phe Leu Leu Ile Ile Ile
245 250 255

Phe Leu Thr Val Leu Leu Leu Lys Leu Arg Lys Arg His Arg Lys His
260 265 270

Thr Gln Gln Arg Ala Ala Ala Leu Ser Leu Ser Thr Leu Ala Ser Pro
275 280 285

Lys Gly Gly Ser Gly Thr Ala Gly Thr Glu Pro Ser Asp Ile Ile Ile
290 295 300

Pro Leu Arg Thr Thr Glu Asn Asn Tyr Cys Pro His Tyr Glu Lys Val
305 310 315 320

Ser Gly Asp Tyr Gly His Pro Val Tyr Ile Val Gln Glu Met Pro Pro
325 330 335

Gln Ser Pro Ala Asn Ile Tyr Tyr Lys Val
340 345

<210> 435
<211> 346
<212> PRT
<213> Homo sapiens

<400> 435
Met Ala Arg Pro Gly Gln Arg Trp Leu Gly Lys Trp Leu Val Ala Met
1 5 10 15

Val Val Trp Ala Leu Cys Arg Leu Ala Thr Pro Leu Ala Lys Asn Leu
20 25 30

Glu Pro Val Ser Trp Ser Ser Leu Asn Pro Lys Phe Leu Ser Gly Lys
35 40 45

Gly Leu Val Ile Tyr Pro Lys Ile Gly Asp Lys Leu Asp Ile Ile Cys
50 55 60

Pro Arg Ala Glu Ala Gly Arg Pro Tyr Glu Tyr Tyr Lys Leu Tyr Leu
65 70 75 80

Val Arg Pro Glu Gln Ala Ala Ala Cys Ser Thr Val Leu Asp Pro Asn
85 90 95

Val Leu Val Thr Cys Asn Arg Pro Glu Gln Glu Ile Arg Phe Thr Ile
100 105 110

Lys Phe Gln Glu Phe Ser Pro Asn Tyr Met Gly Leu Glu Phe Lys Lys
115 120 125

His His Asp Tyr Tyr Ile Thr Ser Thr Ser Asn Gly Ser Leu Glu Gly
130 135 140

Leu Glu Asn Arg Glu Gly Gly Val Cys Arg Thr Arg Thr Met Lys Ile
145 150 155 160

Ile Met Lys Val Gly Gln Asp Pro Asn Ala Val Thr Pro Glu Gln Leu
165 170 175

Thr Thr Ser Arg Pro Ser Lys Glu Ala Asp Asn Thr Val Lys Met Ala
180 185 190

Thr Gln Ala Pro Gly Ser Arg Gly Ser Leu Gly Asp Ser Asp Gly Lys
195 200 205

His Glu Thr Val Asn Gln Glu Glu Lys Ser Gly Pro Gly Ala Ser Gly
210 215 220

584

Gly Ser Ser Gly Asp Pro Asp Gly Phe Phe Asn Ser Lys Val Ala Leu
225 230 235 240

Phe Ala Ala Val Gly Ala Gly Cys Val Ile Phe Leu Leu Ile Ile Ile
245 250 255

Phe Leu Thr Val Leu Leu Leu Lys Leu Arg Lys Arg His Arg Lys His
260 265 270

Thr Gln Gln Arg Ala Ala Ala Leu Ser Leu Ser Thr Leu Ala Ser Pro
275 280 285

Lys Gly Gly Ser Gly Thr Ala Gly Thr Glu Pro Ser Asp Ile Ile Ile
290 295 300

Pro Leu Arg Thr Thr Glu Asn Asn Tyr Cys Pro His Tyr Glu Lys Val
305 310 315 320

Ser Gly Asp Tyr Gly His Pro Val Tyr Ile Val Gln Glu Met Pro Pro
325 330 335

Gln Ser Pro Ala Asn Ile Tyr Tyr Lys Val
340 345

<210> 436
<211> 129
<212> PRT
<213> Homo sapiens

<400> 436
Met Ala Arg Gly Ser Leu Arg Arg Leu Leu Arg Leu Leu Val Leu Gly
1 5 10 15

Leu Trp Leu Ala Leu Leu Arg Ser Val Ala Gly Glu Gln Ala Pro Gly
20 25 30

Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
35 40 45

Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
50 55 60

Leu Gly Cys Ala Ala Ala Pro Pro Ala Pro Phe Arg Leu Leu Trp Pro
65 70 75 80

Ile Leu Gly Gly Ala Leu Ser Leu Thr Phe Val Leu Gly Leu Leu Ser
85 90 95

Gly Phe Leu Val Trp Arg Arg Cys Arg Arg Arg Glu Lys Phe Thr Thr
100 105 110

Pro Ile Glu Glu Thr Gly Gly Glu Gly Cys Pro Ala Val Ala Leu Ile
115 120 125

Gln

<210> 437

<211> 247
<212> PRT
<213> Homo sapiens

<400> 437
Met Leu Arg Arg Arg Gly Ser Pro Gly Met Gly Val His Val Gly Ala
1 5 10 15

Ala Leu Gly Ala Leu Trp Phe Cys Leu Thr Gly Ala Leu Glu Val Gln
20 25 30

Val Pro Glu Asp Pro Val Val Ala Leu Val Gly Thr Asp Ala Thr Leu
35 40 45

Cys Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn
50 55 60

Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Ala
65 70 75 80

Glu Gly Gln Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe
85 90 95

Leu Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val
100 105 110

Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe Val Ser Ile Arg Asp
115 120 125

Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys
130 135 140

Pro Ser Met Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr
145 150 155 160

Val Thr Ile Thr Cys Ser Ser Tyr Arg Gly Tyr Pro Glu Ala Glu Val
165 170 175

Phe Trp Gln Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr
180 185 190

Ser Gln Met Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Val Leu
195 200 205

Arg Val Val Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn
210 215 220

Pro Val Leu Gln Gln Asp Ala His Gly Ser Val Thr Ile Thr Gly Gln
225 230 235 240

Pro Met Thr Phe Pro Pro Glu
245

<210> 438
<211> 346
<212> PRT
<213> Homo sapiens

<400> 438

Met Ala Arg Pro Gly Gln Arg Trp Leu Gly Lys Trp Leu Val Ala Met
1 5 10 15

Val Val Trp Ala Leu Cys Arg Leu Ala Thr Pro Leu Ala Lys Asn Leu
20 25 30

Glu Pro Val Ser Trp Ser Ser Leu Asn Pro Lys Phe Leu Ser Gly Lys
35 40 45

Gly Leu Val Ile Tyr Pro Lys Ile Gly Asp Lys Leu Asp Ile Ile Cys
50 55 60

Pro Arg Ala Glu Ala Gly Arg Pro Tyr Glu Tyr Tyr Lys Leu Tyr Leu
65 70 75 80

Val Arg Pro Glu Gln Ala Ala Ala Cys Ser Thr Val Leu Asp Pro Asn
85 90 95

Val Leu Val Thr Cys Asn Arg Pro Glu Gln Glu Ile Arg Phe Thr Ile
100 105 110

Lys Phe Gln Glu Phe Ser Pro Asn Tyr Met Gly Leu Glu Phe Lys Lys
115 120 125

His His Asp Tyr Tyr Ile Thr Ser Thr Ser Asn Gly Ser Leu Glu Gly
130 135 140

Leu Glu Asn Arg Glu Gly Gly Val Cys Arg Thr Arg Thr Met Lys Ile
145 150 155 160

Ile Met Lys Val Gly Gln Asp Pro Asn Ala Val Thr Pro Glu Gln Leu
165 170 175

Thr Thr Ser Arg Pro Ser Lys Glu Ala Asp Asn Thr Val Lys Met Ala
180 185 190

Thr Gln Ala Pro Gly Ser Arg Gly Ser Leu Gly Asp Ser Asp Gly Lys
195 200 205

His Glu Thr Val Asn Gln Glu Glu Lys Ser Gly Pro Gly Ala Ser Gly
210 215 220

Gly Ser Ser Gly Asp Pro Asp Gly Phe Phe Asn Ser Lys Val Ala Leu
225 230 235 240

Phe Ala Ala Val Gly Ala Gly Cys Val Ile Phe Leu Leu Ile Ile Ile
245 250 255

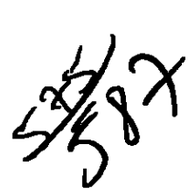
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260 265 270

Thr Gln Gln Arg Ala Ala Ala Leu Ser Leu Ser Thr Leu Ala Ser Pro
275 280 285

Lys Gly Gly Ser Gly Thr Ala Gly Thr Glu Pro Ser Asp Ile Ile Ile
290 295 300

Pro Leu Arg Thr Thr Glu Asn Asn Tyr Cys Pro His Tyr Glu Lys Val
305 310 315 320

Ser Gly Asp Tyr Gly His Pro Val Tyr Ile Val Gln Glu Met Pro Pro



325 330 335

Gln Ser Pro Ala Asn Ile Tyr Tyr Lys Val
340 345

<210> 439
<211> 346
<212> PRT
<213> Homo sapiens

<400> 439
Met Ala Arg Pro Gly Gln Arg Trp Leu Gly Lys Trp Leu Val Ala Met
1 5 10 15

Val Val Trp Ala Leu Cys Arg Leu Ala Thr Pro Leu Ala Lys Asn Leu
20 25 30

Glu Pro Val Ser Trp Ser Ser Leu Asn Pro Lys Phe Leu Ser Gly Lys
35 40 45

Gly Leu Val Ile Tyr Pro Lys Ile Gly Asp Lys Leu Asp Ile Ile Cys
50 55 60

Pro Arg Ala Glu Ala Gly Arg Pro Tyr Glu Tyr Tyr Lys Leu Tyr Leu
65 70 75 80

Val Arg Pro Glu Gln Ala Ala Ala Cys Ser Thr Val Leu Asp Pro Asn
85 90 95

Val Leu Val Thr Cys Asn Arg Pro Glu Gln Glu Ile Arg Phe Thr Ile
100 105 110

Lys Phe Gln Glu Phe Ser Pro Asn Tyr Met Gly Leu Glu Phe Lys Lys
115 120 125

His His Asp Tyr Tyr Ile Thr Ser Thr Ser Asn Gly Ser Leu Glu Gly
130 135 140

Leu Glu Asn Arg Glu Gly Gly Val Cys Arg Thr Arg Thr Met Lys Ile
145 150 155 160

Ile Met Lys Val Gly Gln Asp Pro Asn Ala Val Thr Pro Glu Gln Leu
165 170 175

Thr Thr Ser Arg Pro Ser Lys Glu Ala Asp Asn Thr Val Lys Met Ala
180 185 190

Thr Gln Ala Pro Gly Ser Arg Gly Ser Leu Gly Asp Ser Asp Gly Lys
195 200 205

His Glu Thr Val Asn Gln Glu Glu Lys Ser Gly Pro Gly Ala Ser Gly
210 215 220

Gly Ser Ser Gly Asp Pro Asp Gly Phe Phe Asn Ser Lys Val Ala Leu
225 230 235 240

Phe Ala Ala Val Gly Ala Gly Cys Val Ile Phe Leu Leu Ile Ile Ile
245 250 255



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Phe Leu Thr Val Leu Leu Leu Lys Leu Arg Lys Arg His Arg Lys His
            260                 265                 270

Thr Gln Gln Arg Ala Ala Ala Leu Ser Leu Ser Thr Leu Ala Ser Pro
        275                 280                 285

Lys Gly Gly Ser Gly Thr Ala Gly Thr Glu Pro Ser Asp Ile Ile Ile
    290                 295                 300

Pro Leu Arg Thr Thr Glu Asn Asn Tyr Cys Pro His Tyr Glu Lys Val
305                 310                 315                 320

Ser Gly Asp Tyr Gly His Pro Val Tyr Ile Val Gln Glu Met Pro Pro
                325                 330                 335

Gln Ser Pro Ala Asn Ile Tyr Tyr Lys Val
            340                 345



<210> 440
<211> 319
<212> PRT
<213> Pan troglodytes

<220>
<221> MUTAGEN
<222> (30)
<223> Natural stop codon replaced by Arg

<400> 440
Met Pro Val Phe Ser Leu Gln Asn Asp Glu Val Glu Phe Val Arg Thr
  1               5                  10                  15

Gly Tyr Gly Lys Asp Ile Val Lys Val Leu His Ile Gln Arg Asp Gly
             20                  25                  30

Lys Tyr His Ser Ile Lys Glu Val Ala Thr Ser Val Gln Leu Thr Leu
         35                  40                  45

Ser Ser Lys Lys Asp Tyr Leu His Gly Asp Asn Ser Asp Ile Ile Pro
     50                  55                  60

Thr Asp Thr Ile Lys Asn Thr Val His Val Leu Ala Lys Phe Lys Glu
 65                  70                  75                  80

Asn Glu Pro Ala Asn Ile Asp Gly Ala Met Glu Lys Ala Phe Cys Phe
                 85                  90                  95

Phe Leu Gln Ile Lys Ser Ile Glu Ala Phe Gly Val Asn Ile Cys Glu
            100                 105                 110

His Phe Leu Ser Ser Phe Asn His Val Ile Arg Ala Gln Val Tyr Val
        115                 120                 125

Glu Glu Ile Pro Trp Lys His Leu Glu Lys Asn Gly Val Lys His Val
    130                 135                 140

His Ala Phe Ile His Thr Pro Thr Gly Thr His Phe Gly Glu Val Glu
145                 150                 155                 160
```

Gln Leu Arg Ser Gly Pro Gln Val Ile His Ser Gly Ile Lys Asp Leu
165 170 175

Lys Leu Leu Lys Thr Thr Gln Ser Gly Phe Glu Gly Phe Ile Lys Asp
180 185 190

Gln Phe Thr Thr Leu Pro Glu Val Phe Lys Cys Met Met Arg Thr Leu
195 200 205

Pro Gln Ser Ser Phe Pro Leu Phe Gln Val Leu Ser Met Gly Ser Ser
210 215 220

Leu Met Asp Thr Ile Arg Asp Leu Val Met Glu Lys Ser Ala Gly Pro
225 230 235 240

Tyr Asp Lys Asp Glu Tyr Ser Pro Ser Val Gln Lys Thr Leu Cys Asp
245 250 255

Ile Gln Val Leu Ser Leu Ser Arg Val Pro Ala Ile Glu Asp Met Glu
260 265 270

Ile Ser Leu Pro Asn Ile His Tyr Phe Asn Ile Asp Met Ser Lys Met
275 280 285

Gly Leu Ile Asn Lys Glu Glu Val Leu Leu Pro Leu Asp Asn Pro Tyr
290 295 300

Gly Lys Ile Thr Gly Thr Val Lys Arg Lys Leu Ser Ser Arg Leu
305 310 315

<210> 441
<211> 319
<212> PRT
<213> Pan troglodytes

<220>
<221> MUTAGEN
<222> (30)
<223> Natural stop codon replaced by Arg

<400> 441
Met Pro Val Phe Ser Leu Gln Asn Asp Glu Val Glu Phe Val Arg Thr
1 5 10 15

Gly Tyr Gly Lys Asp Ile Val Lys Val Leu His Ile Gln Arg Asp Gly
20 25 30

Lys Tyr His Ser Ile Lys Glu Val Ala Thr Ser Val Gln Leu Thr Leu
35 40 45

Ser Ser Lys Lys Asp Tyr Leu His Gly Asp Asn Ser Asp Ile Ile Pro
50 55 60

Thr Asp Thr Ile Lys Asn Thr Val His Val Leu Ala Lys Phe Lys Glu
65 70 75 80

Asn Glu Pro Ala Asn Ile Asp Gly Ala Met Glu Lys Ala Phe Cys Phe
85 90 95

Phe Leu Gln Ile Lys Ser Ile Glu Ala Phe Gly Val Asn Ile Cys Glu
100 105 110

His Phe Leu Ser Ser Phe Asn His Val Ile Arg Ala Gln Val Tyr Val
115 120 125

Glu Glu Ile Pro Trp Lys His Leu Glu Lys Asn Gly Val Lys His Val
130 135 140

His Ala Phe Ile His Thr Pro Thr Gly Thr His Phe Gly Glu Val Glu
145 150 155 160

Gln Leu Arg Ser Gly Pro Gln Val Ile His Ser Gly Ile Lys Asp Leu
165 170 175

Lys Leu Leu Lys Thr Thr Gln Ser Gly Phe Glu Gly Phe Ile Lys Asp
180 185 190

Gln Phe Thr Thr Leu Pro Glu Val Phe Lys Cys Met Met Arg Thr Leu
195 200 205

Pro Gln Ser Ser Phe Pro Leu Phe Gln Val Leu Ser Met Gly Ser Ser
210 215 220

Leu Met Asp Thr Ile Arg Asp Leu Val Met Glu Lys Ser Ala Gly Pro
225 230 235 240

Tyr Asp Lys Asp Glu Tyr Ser Pro Ser Val Gln Lys Thr Leu Cys Asp
245 250 255

Ile Gln Val Leu Ser Leu Ser Arg Val Pro Ala Ile Glu Asp Met Glu
260 265 270

Ile Ser Leu Pro Asn Ile His Tyr Phe Asn Ile Asp Met Ser Lys Met
275 280 285

Gly Leu Ile Asn Lys Glu Glu Val Leu Leu Pro Leu Asp Asn Pro Tyr
290 295 300

Gly Lys Ile Thr Gly Thr Val Lys Arg Lys Leu Ser Ser Arg Leu
305 310 315

<210> 442
<211> 394
<212> PRT
<213> Homo sapiens

<220>
<221> MUTAGEN
<222> (104)
<223> Natural stop codon replaced by Arg

<220>
<221> MUTAGEN
<222> (277)
<223> Natural stop codon replaced by Arg

<400> 442
Met Glu Lys Phe Ile Trp Tyr Leu Val His Leu Tyr Thr Glu Met Thr

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 1               5                  10                  15

Lys Ser Ser Pro Ser Cys Arg Leu Val Ala Ser Cys Gln Thr Val Ala
            20                  25                  30

Lys Glu Leu Gly Glu Asn Ser Leu Gly Tyr Gly Pro Gly His Tyr Leu
        35                  40                  45

Leu Phe Gly Cys Arg Asp Ala Phe Gly Cys Pro Met Pro Gly Leu Phe
    50                  55                  60

His Leu Leu Gln Asp Gln Met Ile Gly Ser Leu His Thr Asp Ser Leu
65                  70                  75                  80

Pro Asn Asp Glu Val Glu Phe Val Arg Thr Gly Tyr Gly Lys Glu Met
                85                  90                  95

Val Lys Val Leu His Ile Gln Arg Asp Gly Lys Tyr His Ser Ile Lys
            100                 105                 110

Glu Val Ala Thr Ser Val Gln Leu Thr Leu Ser Ser Lys Lys Asp Tyr
        115                 120                 125

Leu His Gly Asp Asn Ser Asp Ile Ile Pro Thr Asp Thr Ile Lys Asn
    130                 135                 140

Thr Val His Val Leu Ala Lys Phe Lys Glu Asn Asp Pro Ala Asn Ile
145                 150                 155                 160

Asp Gly Ala Met Glu Lys Ala Phe Cys Phe Phe Leu Gln Ile Lys Ser
                165                 170                 175

Ile Glu Ala Phe Gly Val Asn Ile Cys Glu His Phe Leu Ser Ser Phe
            180                 185                 190

Asn His Val Ile Arg Ala Gln Val Tyr Met Glu Glu Ile Pro Trp Lys
        195                 200                 205

His Leu Gly Lys Asn Gly Val Lys His Val His Ala Phe Ile His Thr
    210                 215                 220

Pro Thr Gly Thr His Phe Cys Glu Val Glu Gln Leu Arg Ser Gly Pro
225                 230                 235                 240

Gln Val Ile His Ser Gly Ile Lys Asp Leu Lys Val Leu Lys Thr Thr
                245                 250                 255

Gln Ser Gly Phe Glu Gly Phe Ile Lys Asp Gln Phe Thr Thr Leu Pro
            260                 265                 270

Glu Val Lys Asp Arg Cys Phe Ala Thr Gln Val Tyr Cys Lys Trp Arg
        275                 280                 285

Tyr His Gln Cys Arg Asp Val Asp Phe Lys Ala Thr Trp Asp Thr Ile
    290                 295                 300

Arg Asp Leu Val Met Glu Lys Ser Ala Gly Pro Tyr Asp Lys Gly Glu
305                 310                 315                 320

Tyr Leu Thr Ser Val Gln Lys Thr Leu Cys Asp Ile Gln Val Leu Ser
                325                 330                 335
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Leu Ser Arg Val Pro Ala Ile Glu Asp Met Glu Ile Ser Leu Pro Asn
340 345 350

Ile His Tyr Phe Asn Ile Asp Met Ser Lys Met Gly Leu Ile Asn Lys
355 360 365

Glu Glu Val Leu Leu Pro Leu Asp Asn Pro Tyr Gly Lys Ile Thr Gly
370 375 380

Thr Val Lys Arg Lys Leu Ser Ser Arg Leu
385 390

<210> 443
<211> 394
<212> PRT
<213> Homo sapiens

<220>
<221> MUTAGEN
<222> (104)
<223> Natural stop codon replaced by Arg

<220>
<221> MUTAGEN
<222> (277)
<223> Natural stop codon replaced by Arg

<400> 443
Met Glu Lys Phe Ile Trp Tyr Leu Val His Leu Tyr Thr Glu Met Thr
1 5 10 15

Lys Ser Ser Pro Ser Cys Arg Leu Val Ala Ser Cys Gln Thr Val Ala
20 25 30

Lys Glu Leu Gly Glu Asn Ser Leu Gly Tyr Gly Pro Gly His Tyr Leu
35 40 45

Leu Phe Gly Cys Arg Asp Ala Phe Gly Cys Pro Met Pro Gly Leu Phe
50 55 60

His Leu Leu Gln Asp Gln Met Ile Gly Ser Leu His Thr Asp Ser Leu
65 70 75 80

Pro Asn Asp Glu Val Glu Phe Val Arg Thr Gly Tyr Gly Lys Glu Met
85 90 95

Val Lys Val Leu His Ile Gln Arg Asp Gly Lys Tyr His Ser Ile Lys
100 105 110

Glu Val Ala Thr Ser Val Gln Leu Thr Leu Ser Ser Lys Lys Asp Tyr
115 120 125

Leu His Gly Asp Asn Ser Asp Ile Ile Pro Thr Asp Thr Ile Lys Asn
130 135 140

Thr Val His Val Leu Ala Lys Phe Lys Glu Asn Asp Pro Ala Asn Ile
145 150 155 160

Asp Gly Ala Met Glu Lys Ala Phe Cys Phe Phe Leu Gln Ile Lys Ser
165 170 175

Ile Glu Ala Phe Gly Val Asn Ile Cys Glu His Phe Leu Ser Ser Phe
180 185 190

Asn His Val Ile Arg Ala Gln Val Tyr Met Glu Glu Ile Pro Trp Lys
195 200 205

His Leu Gly Lys Asn Gly Val Lys His Val His Ala Phe Ile His Thr
210 215 220

Pro Thr Gly Thr His Phe Cys Glu Val Glu Gln Leu Arg Ser Gly Pro
225 230 235 240

Gln Val Ile His Ser Gly Ile Lys Asp Leu Lys Val Leu Lys Thr Thr
245 250 255

Gln Ser Gly Phe Glu Gly Phe Ile Lys Asp Gln Phe Thr Thr Leu Pro
260 265 270

Glu Val Lys Asp Arg Cys Phe Ala Thr Gln Val Tyr Cys Lys Trp Arg
275 280 285

Tyr His Gln Cys Arg Asp Val Asp Phe Lys Ala Thr Trp Asp Thr Ile
290 295 300

Arg Asp Leu Val Met Glu Lys Ser Ala Gly Pro Tyr Asp Lys Gly Glu
305 310 315 320

Tyr Leu Thr Ser Val Gln Lys Thr Leu Cys Asp Ile Gln Val Leu Ser
325 330 335

Leu Ser Arg Val Pro Ala Ile Glu Asp Met Glu Ile Ser Leu Pro Asn
340 345 350

Ile His Tyr Phe Asn Ile Asp Met Ser Lys Met Gly Leu Ile Asn Lys
355 360 365

Glu Glu Val Leu Leu Pro Leu Asp Asn Pro Tyr Gly Lys Ile Thr Gly
370 375 380

Thr Val Lys Arg Lys Leu Ser Ser Arg Leu
385 390

<210> 444
<211> 304
<212> PRT
<213> Papio hamadryas

<400> 444
Met Ala Asp Tyr His Asn Asn Tyr Lys Lys Asn Asp Glu Leu Glu Phe
1 5 10 15

Val Arg Thr Gly Tyr Gly Lys Asp Met Val Lys Val Leu His Ile Gln
20 25 30

Arg Asp Gly Lys Tyr His Ser Ile Lys Glu Val Ala Thr Ser Val Gln
35 40 45

Leu Thr Leu Ser Ser Lys Lys Asp Tyr Leu His Gly Asp Asn Ser Asp
50 55 60

Ile Ile Pro Thr Asp Thr Ile Lys Asn Thr Val His Val Leu Ala Lys
65 70 75 80

Phe Lys Gly Ile Lys Ser Ile Glu Ala Phe Gly Val Asn Ile Cys Glu
85 90 95

Tyr Phe Leu Ser Ser Phe Asn His Val Ile Arg Ala Gln Val Tyr Val
100 105 110

Glu Glu Ile Pro Trp Lys Arg Leu Glu Lys Asn Gly Val Lys His Val
115 120 125

His Ala Phe Ile His Thr Pro Thr Gly Thr His Phe Cys Glu Val Glu
130 135 140

Gln Leu Arg Ser Gly Pro Pro Val Ile His Ser Gly Ile Lys Asp Leu
145 150 155 160

Lys Val Leu Lys Thr Thr Gln Ser Gly Phe Glu Gly Phe Ile Lys Asp
165 170 175

Gln Phe Thr Thr Leu Pro Glu Val Lys Asp Arg Cys Phe Ala Thr Gln
180 185 190

Val Tyr Cys Lys Trp Arg Tyr His Gln Cys Arg Asp Val Asp Phe Glu
195 200 205

Ala Thr Trp Gly Thr Ile Arg Asp Leu Val Leu Glu Lys Phe Ala Gly
210 215 220

Pro Tyr Asp Lys Gly Glu Tyr Ser Pro Ser Val Gln Lys Thr Leu Tyr
225 230 235 240

Asp Ile Gln Val Leu Ser Leu Ser Arg Val Pro Glu Ile Glu Asp Met
245 250 255

Glu Ile Ser Leu Pro Asn Ile His Tyr Phe Asn Ile Asp Met Ser Lys
260 265 270

Met Gly Leu Ile Asn Lys Glu Glu Val Leu Leu Pro Leu Asp Asn Pro
275 280 285

Tyr Gly Lys Ile Thr Gly Thr Val Lys Arg Lys Leu Ser Ser Arg Leu
290 295 300

<210> 445
<211> 304
<212> PRT
<213> Papio hamadryas

<400> 445
Met Ala Asp Tyr His Asn Asn Tyr Lys Lys Asn Asp Glu Leu Glu Phe
1 5 10 15

Val Arg Thr Gly Tyr Gly Lys Asp Met Val Lys Val Leu His Ile Gln
20 25 30

Arg Asp Gly Lys Tyr His Ser Ile Lys Glu Val Ala Thr Ser Val Gln
35 40 45

Leu Thr Leu Ser Ser Lys Lys Asp Tyr Leu His Gly Asp Asn Ser Asp
50 55 60

Ile Ile Pro Thr Asp Thr Ile Lys Asn Thr Val His Val Leu Ala Lys
65 70 75 80

Phe Lys Gly Ile Lys Ser Ile Glu Ala Phe Gly Val Asn Ile Cys Glu
85 90 95

Tyr Phe Leu Ser Ser Phe Asn His Val Ile Arg Ala Gln Val Tyr Val
100 105 110

Glu Glu Ile Pro Trp Lys Arg Leu Glu Lys Asn Gly Val Lys His Val
115 120 125

His Ala Phe Ile His Thr Pro Thr Gly Thr His Phe Cys Glu Val Glu
130 135 140

Gln Leu Arg Ser Gly Pro Pro Val Ile His Ser Gly Ile Lys Asp Leu
145 150 155 160

Lys Val Leu Lys Thr Thr Gln Ser Gly Phe Glu Gly Phe Ile Lys Asp
165 170 175

Gln Phe Thr Thr Leu Pro Glu Val Lys Asp Arg Cys Phe Ala Thr Gln
180 185 190

Val Tyr Cys Lys Trp Arg Tyr His Gln Cys Arg Asp Val Asp Phe Glu
195 200 205

Ala Thr Trp Gly Thr Ile Arg Asp Leu Val Leu Glu Lys Phe Ala Gly
210 215 220

Pro Tyr Asp Lys Gly Glu Tyr Ser Pro Ser Val Gln Lys Thr Leu Tyr
225 230 235 240

Asp Ile Gln Val Leu Ser Leu Ser Arg Val Pro Glu Ile Glu Asp Met
245 250 255

Glu Ile Ser Leu Pro Asn Ile His Tyr Phe Asn Ile Asp Met Ser Lys
260 265 270

Met Gly Leu Ile Asn Lys Glu Glu Val Leu Leu Pro Leu Asp Asn Pro
275 280 285

Tyr Gly Lys Ile Thr Gly Thr Val Lys Arg Lys Leu Ser Ser Arg Leu
290 295 300

<210> 446
<211> 394
<212> PRT
<213> Homo sapiens

<220>
<221> MUTAGEN
<222> (104)
<223> Natural stop codon replaced by Arg

<220>
<221> MUTAGEN
<222> (277)
<223> Natural stop codon replaced by Arg

<400> 446
Met Glu Lys Phe Ile Trp Tyr Leu Val His Leu Tyr Thr Glu Met Thr
1 5 10 15

Lys Ser Ser Pro Ser Cys Arg Leu Val Ala Ser Cys Gln Thr Val Ala
20 25 30

Lys Glu Leu Gly Glu Asn Ser Leu Gly Tyr Gly Pro Gly His Tyr Leu
35 40 45

Leu Phe Gly Cys Arg Asp Ala Phe Gly Cys Pro Met Pro Gly Leu Phe
50 55 60

His Leu Leu Gln Asp Gln Met Ile Gly Ser Leu His Thr Asp Ser Leu
65 70 75 80

Pro Asn Asp Glu Val Glu Phe Val Arg Thr Gly Tyr Gly Lys Glu Met
85 90 95

Val Lys Val Leu His Ile Gln Arg Asp Gly Lys Tyr His Ser Ile Lys
100 105 110

Glu Val Ala Thr Ser Val Gln Leu Thr Leu Ser Ser Lys Lys Asp Tyr
115 120 125

Leu His Gly Asp Asn Ser Asp Ile Ile Pro Thr Asp Thr Ile Lys Asn
130 135 140

Thr Val His Val Leu Ala Lys Phe Lys Glu Asn Asp Pro Ala Asn Ile
145 150 155 160

Asp Gly Ala Met Glu Lys Ala Phe Cys Phe Phe Leu Gln Ile Lys Ser
165 170 175

Ile Glu Ala Phe Gly Val Asn Ile Cys Glu His Phe Leu Ser Ser Phe
180 185 190

Asn His Val Ile Arg Ala Gln Val Tyr Met Glu Glu Ile Pro Trp Lys
195 200 205

His Leu Gly Lys Asn Gly Val Lys His Val His Ala Phe Ile His Thr
210 215 220

Pro Thr Gly Thr His Phe Cys Glu Val Glu Gln Leu Arg Ser Gly Pro
225 230 235 240

Gln Val Ile His Ser Gly Ile Lys Asp Leu Lys Val Leu Lys Thr Thr
245 250 255

Gln Ser Gly Phe Glu Gly Phe Ile Lys Asp Gln Phe Thr Thr Leu Pro
260 265 270

Glu Val Lys Asp Arg Cys Phe Ala Thr Gln Val Tyr Cys Lys Trp Arg
275 280 285

Tyr His Gln Cys Arg Asp Val Asp Phe Lys Ala Thr Trp Asp Thr Ile
290 295 300

Arg Asp Leu Val Met Glu Lys Ser Ala Gly Pro Tyr Asp Lys Gly Glu
305 310 315 320

Tyr Leu Thr Ser Val Gln Lys Thr Leu Cys Asp Ile Gln Val Leu Ser
325 330 335

Leu Ser Arg Val Pro Ala Ile Glu Asp Met Glu Ile Ser Leu Pro Asn
340 345 350

Ile His Tyr Phe Asn Ile Asp Met Ser Lys Met Gly Leu Ile Asn Lys
355 360 365

Glu Glu Val Leu Leu Pro Leu Asp Asn Pro Tyr Gly Lys Ile Thr Gly
370 375 380

Thr Val Lys Arg Lys Leu Ser Ser Arg Leu
385 390

<210> 447
<211> 319
<212> PRT
<213> Pan troglodytes

<220>
<221> MUTAGEN
<222> (30)
<223> Natural stop codon replaced by Arg

<400> 447
Met Pro Val Phe Ser Leu Gln Asn Asp Glu Val Glu Phe Val Arg Thr
1 5 10 15

Gly Tyr Gly Lys Asp Ile Val Lys Val Leu His Ile Gln Arg Asp Gly
20 25 30

Lys Tyr His Ser Ile Lys Glu Val Ala Thr Ser Val Gln Leu Thr Leu
35 40 45

Ser Ser Lys Lys Asp Tyr Leu His Gly Asp Asn Ser Asp Ile Ile Pro
50 55 60

Thr Asp Thr Ile Lys Asn Thr Val His Val Leu Ala Lys Phe Lys Glu
65 70 75 80

Asn Glu Pro Ala Asn Ile Asp Gly Ala Met Glu Lys Ala Phe Cys Phe
85 90 95

Phe Leu Gln Ile Lys Ser Ile Glu Ala Phe Gly Val Asn Ile Cys Glu
100 105 110

His Phe Leu Ser Ser Phe Asn His Val Ile Arg Ala Gln Val Tyr Val
115 120 125

Glu Glu Ile Pro Trp Lys His Leu Glu Lys Asn Gly Val Lys His Val
130 135 140

His Ala Phe Ile His Thr Pro Thr Gly Thr His Phe Gly Glu Val Glu
145 150 155 160

Gln Leu Arg Ser Gly Pro Gln Val Ile His Ser Gly Ile Lys Asp Leu
165 170 175

Lys Leu Leu Lys Thr Thr Gln Ser Gly Phe Glu Gly Phe Ile Lys Asp
180 185 190

Gln Phe Thr Thr Leu Pro Glu Val Phe Lys Cys Met Met Arg Thr Leu
195 200 205

Pro Gln Ser Ser Phe Pro Leu Phe Gln Val Leu Ser Met Gly Ser Ser
210 215 220

Leu Met Asp Thr Ile Arg Asp Leu Val Met Glu Lys Ser Ala Gly Pro
225 230 235 240

Tyr Asp Lys Asp Glu Tyr Ser Pro Ser Val Gln Lys Thr Leu Cys Asp
245 250 255

Ile Gln Val Leu Ser Leu Ser Arg Val Pro Ala Ile Glu Asp Met Glu
260 265 270

Ile Ser Leu Pro Asn Ile His Tyr Phe Asn Ile Asp Met Ser Lys Met
275 280 285

Gly Leu Ile Asn Lys Glu Glu Val Leu Leu Pro Leu Asp Asn Pro Tyr
290 295 300

Gly Lys Ile Thr Gly Thr Val Lys Arg Lys Leu Ser Ser Arg Leu
305 310 315

<210> 448
<211> 304
<212> PRT
<213> Papio hamadryas

<400> 448
Met Ala Asp Tyr His Asn Asn Tyr Lys Lys Asn Asp Glu Leu Glu Phe
1 5 10 15

Val Arg Thr Gly Tyr Gly Lys Asp Met Val Lys Val Leu His Ile Gln
20 25 30

Arg Asp Gly Lys Tyr His Ser Ile Lys Glu Val Ala Thr Ser Val Gln
35 40 45

Leu Thr Leu Ser Ser Lys Lys Asp Tyr Leu His Gly Asp Asn Ser Asp
50 55 60

Ile Ile Pro Thr Asp Thr Ile Lys Asn Thr Val His Val Leu Ala Lys
65 70 75 80

Phe Lys Gly Ile Lys Ser Ile Glu Ala Phe Gly Val Asn Ile Cys Glu
85 90 95

Tyr Phe Leu Ser Ser Phe Asn His Val Ile Arg Ala Gln Val Tyr Val
100 105 110

Glu Glu Ile Pro Trp Lys Arg Leu Glu Lys Asn Gly Val Lys His Val
115 120 125

His Ala Phe Ile His Thr Pro Thr Gly Thr His Phe Cys Glu Val Glu
130 135 140

Gln Leu Arg Ser Gly Pro Pro Val Ile His Ser Gly Ile Lys Asp Leu
145 150 155 160

Lys Val Leu Lys Thr Thr Gln Ser Gly Phe Glu Gly Phe Ile Lys Asp
165 170 175

Gln Phe Thr Thr Leu Pro Glu Val Lys Asp Arg Cys Phe Ala Thr Gln
180 185 190

Val Tyr Cys Lys Trp Arg Tyr His Gln Cys Arg Asp Val Asp Phe Glu
195 200 205

Ala Thr Trp Gly Thr Ile Arg Asp Leu Val Leu Glu Lys Phe Ala Gly
210 215 220

Pro Tyr Asp Lys Gly Glu Tyr Ser Pro Ser Val Gln Lys Thr Leu Tyr
225 230 235 240

Asp Ile Gln Val Leu Ser Leu Ser Arg Val Pro Glu Ile Glu Asp Met
245 250 255

Glu Ile Ser Leu Pro Asn Ile His Tyr Phe Asn Ile Asp Met Ser Lys
260 265 270

Met Gly Leu Ile Asn Lys Glu Glu Val Leu Leu Pro Leu Asp Asn Pro
275 280 285

Tyr Gly Lys Ile Thr Gly Thr Val Lys Arg Lys Leu Ser Ser Arg Leu
290 295 300

<210> 449
<211> 23
<212> DNA
<213> Homo sapiens

<400> 449
caccaaatgc tgcacagaat cct 23

<210> 450
<211> 20
<212> DNA
<213> Homo sapiens

<400> 450
acctgaccta caggaaagag 20

<210> 451
<211> 23
<212> DNA
<213> Homo sapiens

<400> 451
caccaaatgc tgcacagaat cct 23

<210> 452
<211> 20
<212> DNA
<213> Homo sapiens

<400> 452
acctgaccta caggaaagag 20

<210> 453
<211> 23
<212> DNA
<213> Homo sapiens

<400> 453
caccaaatgc tgcacagaat cct 23

<210> 454
<211> 20
<212> DNA
<213> Homo sapiens

<400> 454
acctgaccta caggaaagag 20

<210> 455
<211> 20
<212> DNA
<213> Homo sapiens

<400> 455
catacaaact taagagtcca 20

<210> 456
<211> 67
<212> DNA
<213> Homo sapiens

<400> 456
ctttaaatcg atgagcaacc tcactcttgt gtgcatctct cttatccaaa gaaccggaat 60
aagccga 67

<210> 457
<211> 20
<212> DNA
<213> Homo sapiens

<400> 457
catacaaact taagagtcca 20

<210> 458
<211> 67
<212> DNA
<213> Homo sapiens

<400> 458
ctttaaatcg atgagcaacc tcactcttgt gtgcatctct cttatccaaa gaaccggaat 60
aagccga 67

<210> 459
<211> 27
<212> DNA
<213> Homo sapiens

<400> 459
gttagcagag tagcagggct ttcggct 27

<210> 460
<211> 95
<212> DNA
<213> Homo sapiens

<400> 460
caagcaatga attccttagc agcttgacct tccaagtaag aagaaacatc agaagtgaaa 60
gtaccttcac cgtgtctctt atccaaagaa ccgga 95

<210> 461
<211> 103
<212> DNA
<213> Homo sapiens

<400> 461
gagcgcaagc ttccgccatc atgaaggtct ccgtggctgc cctctcctgc ctcatgcttg 60
ttactgccct tggatcccag gccagcccca agatggtgca agg 103

<210> 462
<211> 57
<212> DNA
<213> Homo sapiens

<400> 462
agtcccatcg atgagcaacc tcactcttgt gtgcatcatg ccgcctcagc actttgc 57

<210> 463
<211> 103
<212> DNA
<213> Homo sapiens

<400> 463
gagcgcaagc ttccgccatc atgtggtggc gcctgtggtg gctgctgctg ctgctgctgc 60
tgctgtggcc catggtgtgg gccagcccca agatggtgca agg 103

<210> 464
<211> 57
<212> DNA
<213> Homo sapiens

<400> 464
agtcccatcg atgagcaacc tcactcttgt gtgcatcatg ccgcctcagc actttgc 57

<210> 465
<211> 104
<212> DNA
<213> Homo sapiens

<400> 465
tcccccgggg ccgccaccat gaaggtctcc gtggctgccc tctcctgcct catgcttgtt 60
actgcccttg gatcccaggc ccacggtgaa ggtactttca cttc 104

<210> 466

<211> 49
<212> DNA
<213> Homo sapiens

<400> 466
cgattgatca ttattataag cctaaggcag cttgacttgc agcaacaag 49

<210> 467
<211> 38
<212> DNA
<213> Homo sapiens

<400> 467
aggagcgtcg acaaaagaag ccccaagatg gtgcaagg 38

<210> 468
<211> 26
<212> DNA
<213> Homo sapiens

<400> 468
ttataagcct aaggcagctt gacttg 26

<210> 469
<211> 115
<212> DNA
<213> Homo sapiens

<400> 469
gagcgcggat ccaagcttcc gccatcatgc gccccacctg ggcctggtgg ctgttcctgg 60
tgctgctgct ggccctgtgg gccccgccc gcggcagccc caagatggtg caagg 115

<210> 470
<211> 57
<212> DNA
<213> Homo sapiens

<400> 470
agtcccatcg atgagcaacc tcactcttgt gtgcatcatg ccgcctcagc actttgc 57

<210> 471
<211> 29
<212> DNA
<213> Homo sapiens

<400> 471
gagcgcggat ccaagcttcc gccatcatg 29

<210> 472
<211> 26
<212> DNA
<213> Homo sapiens

<400> 472
ctttaaatcg atgagcaacc tcactc 26

<210> 473
<211> 109
<212> DNA
<213> Homo sapiens

<400> 473
gagcgcggat ccaagcttcc gccatcatgt ggtggcgcct gtggtggctg ctgctgctgc 60
tgctgctgct gtggcccatg gtgtgggcca gccccaagat ggtgcaagg 109

<210> 474
<211> 38
<212> DNA
<213> Homo sapiens

<400> 474
gtcgtcggta ccttataagc ctaaggcagc ttgacttg 38

<210> 475
<211> 80
<212> DNA
<213> Homo sapiens

<400> 475
gagcgcggat ccaagcttcc gccatcatgt ggtggcgcct gtggtggctg ctgctgctgc 60
tgctgctgct gtggcccatg 80

<210> 476
<211> 57
<212> DNA
<213> Homo sapiens

<400> 476
agtcccatcg atgagcaacc tcactcttgt gtgcatcatg ccgcctcagc actttgc 57

<210> 477
<211> 80
<212> DNA
<213> Homo sapiens

<400> 477
gagcgcggat ccaagcttcc gccatcatgt ggtggcgcct gtggtggctg ctgctgctgc 60
tgctgctgct gtggcccatg 80

<210> 478
<211> 57
<212> DNA
<213> Homo sapiens

<400> 478
agtcccatcg atgagcaacc tcactcttgt gtgcatcatg ccgcctcagc actttgc 57

<210> 479
<211> 80
<212> DNA
<213> Homo sapiens

<400> 479
aagctgcctt aggcttaagc cccaagatgg tgcaagggtc tggctgcttt gggaggaaga 60
tggaccggat cagctcctcc 80

<210> 480
<211> 64
<212> DNA
<213> Homo sapiens

<400> 480

gcaccgggcg cgccttaatg ccgcctcagc actttgcagc ccaggccact ggaggagctg 60
atcc 64

<210> 481
<211> 37
<212> DNA
<213> Homo sapiens

<400> 481
aggagcgtcg acaaaagaag ccccaagatg gtgcaag 37

<210> 482
<211> 56
<212> DNA
<213> Homo sapiens

<400> 482
cgcgcatcga tgagcaacct cactcttgtg tgcatcatgc cgcctcagca ctttgc 56

<210> 483
<211> 670
<212> PRT
<213> Homo sapiens

<400> 483

Met	Trp	Trp	Arg	Leu	Trp	Trp	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Trp
1				5				10					15		
Pro	Met	Val	Trp	Ala	Ser	Pro	Lys	Met	Val	Gln	Gly	Ser	Gly	Cys	Phe
			20					25					30		
Gly	Arg	Lys	Met	Asp	Arg	Ile	Ser	Ser	Ser	Ser	Gly	Leu	Gly	Cys	Lys
		35					40					45			
Val	Leu	Arg	Arg	His	Ser	Pro	Lys	Met	Val	Gln	Gly	Ser	Gly	Cys	Phe
	50					55					60				
Gly	Arg	Lys	Met	Asp	Arg	Ile	Ser	Ser	Ser	Ser	Gly	Leu	Gly	Cys	Lys
65					70					75					80
Val	Leu	Arg	Arg	His	Asp	Ala	His	Lys	Ser	Glu	Val	Ala	His	Arg	Phe
				85					90					95	
Lys	Asp	Leu	Gly	Glu	Glu	Asn	Phe	Lys	Ala	Leu	Val	Leu	Ile	Ala	Phe
			100					105					110		
Ala	Gln	Tyr	Leu	Gln	Gln	Cys	Pro	Phe	Glu	Asp	His	Val	Lys	Leu	Val
		115					120					125			
Asn	Glu	Val	Thr	Glu	Phe	Ala	Lys	Thr	Cys	Val	Ala	Asp	Glu	Ser	Ala
	130					135					140				
Glu	Asn	Cys	Asp	Lys	Ser	Leu	His	Thr	Leu	Phe	Gly	Asp	Lys	Leu	Cys
145					150					155					160
Thr	Val	Ala	Thr	Leu	Arg	Glu	Thr	Tyr	Gly	Glu	Met	Ala	Asp	Cys	Cys
				165					170					175	
Ala	Lys	Gln	Glu	Pro	Glu	Arg	Asn	Glu	Cys	Phe	Leu	Gln	His	Lys	Asp
			180					185					190		

Asp Asn Pro Asn Leu Pro Arg Leu Val Arg Pro Glu Val Asp Val Met
195 200 205

Cys Thr Ala Phe His Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu
210 215 220

Tyr Glu Ile Ala Arg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu
225 230 235 240

Phe Phe Ala Lys Arg Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala
245 250 255

Ala Asp Lys Ala Ala Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp
260 265 270

Glu Gly Lys Ala Ser Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu
275 280 285

Gln Lys Phe Gly Glu Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu
290 295 300

Ser Gln Arg Phe Pro Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val
305 310 315 320

Thr Asp Leu Thr Lys Val His Thr Glu Cys Cys His Gly Asp Leu Leu
325 330 335

Glu Cys Ala Asp Asp Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn
340 345 350

Gln Asp Ser Ile Ser Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu
355 360 365

Leu Glu Lys Ser His Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro
370 375 380

Ala Asp Leu Pro Ser Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val
385 390 395 400

Cys Lys Asn Tyr Ala Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu
405 410 415

Tyr Glu Tyr Ala Arg Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu
420 425 430

Arg Leu Ala Lys Thr Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala
435 440 445

Ala Asp Pro His Glu Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro
450 455 460

Leu Val Glu Glu Pro Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe
465 470 475 480

Glu Gln Leu Gly Glu Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr
485 490 495

Thr Lys Lys Val Pro Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser
500 505 510

Arg Asn Leu Gly Lys Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala
515 520 525

Lys Arg Met Pro Cys Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln
530 535 540

Leu Cys Val Leu His Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys
545 550 555 560

Cys Cys Thr Glu Ser Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu
565 570 575

Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe
580 585 590

Thr Phe His Ala Asp Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile
595 600 605

Lys Lys Gln Thr Ala Leu Val Glu Leu Val Lys His Lys Pro Lys Ala
610 615 620

Thr Lys Glu Gln Leu Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val
625 630 635 640

Glu Lys Cys Cys Lys Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu
645 650 655

Gly Lys Lys Leu Val Ala Ala Ser Gln Ala Ala Leu Gly Leu
660 665 670

<210> 484
<211> 96
<212> DNA
<213> Homo sapiens

<400> 484
agccccaaga tggtgcaagg gtctggctgc tttgggagga agatggaccg gatcagctcc 60
tccagtggcc tgggctgcaa agtgctgagg cggcat 96

<210> 485
<211> 32
<212> PRT
<213> Homo sapiens

<400> 485
Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met Asp
1 5 10 15

Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His
20 25 30

<210> 486
<211> 95
<212> DNA
<213> Homo sapiens

<400> 486
agccccaaga tggtgcaagg gtctggctgc tttgggagga agatggaccg gatcagctcc 60

tccagtggct gggctgcaaa gtgctgaggc ggcat 95

<210> 487
<211> 40
<212> DNA
<213> Homo sapiens

<400> 487
ccttgcacca tcttggggct atgccgcctc agcactttgc 40

<210> 488
<211> 40
<212> DNA
<213> Homo sapiens

<400> 488
gcaaagtgct gaggcggcat agccccaaga tggtgcaagg 40

<210> 489
<211> 57
<212> DNA
<213> Homo sapiens

<400> 489
agtcccatcg atgagcaacc tcactcttgt gtgcatcatg ccgcctcagc actttgc 57

PROTEÍNAS DE FUSIÓN DE ALBÚMINA

REFERENCIA A LISTADO DE SECUENCIAS EN CD

Esta solicitud se refiere a un "listado de secuencias" divulgado más adelante, el cual se suministra como un documento electrónico en tres discos compactos idénticos (CD-R), rotulados "Copia 1," "Copia 2," y "Copia 3." Estos CDs contienen el archivo "PF612PCT SL.txt" (929.048 bytes, creado el 7 de Febrero de 2005), el cual se incorpora como referencia en su totalidad.

FUNDAMENTO DEL INVENTO

El invento generalmente se refiere a proteínas terapéuticas (incluyendo, pero sin limitar a por lo menos un polipéptido, anticuerpo, péptido, o fragmento y variante del mismo) fusionadas a albúmina o fragmentos o variantes de albúmina. El invento incluye polinucleótidos que codifican proteínas terapéuticas de fusión de albúmina, composiciones, composiciones farmacéuticas, fórmulaciones y kits. El invento también contempla células huésped transformadas con los polinucleótidos que codifican proteínas terapéuticas de fusión de albúmina, así como también métodos para preparación de proteínas de fusión de albúmina del invento utilizando estos polinucleótidos, y/o células huésped.

La albúmina sérica humana (HSA, o HA), una proteína de 585 aminoácidos en su forma madura (como se muestra en la Figura 1 (SEQ ID NO:1)), es responsable de una proporción significativa de la presión osmótica del suero y también funciona como un vehículo de ligandos endógenos y exógenos. Actual HA para uso clínico se produce mediante extracción a partir de sangre humana. La producción de HA recombinante (rHA) en microorganismos se ha divulgado en EP 330 451 u EP 361 991.

Las proteínas terapéuticas en su estado nativo o cuando se producen de manera recombinante, tales como interferones y hormonas de crecimiento, típicamente son moléculas lábiles que exhiben vidas en almacenamiento cortas, particularmente cuando se fórmulan en soluciones acuosas. La inestabilidad de estas moléculas cuando se fórmulan para administración impone que muchas de las moléculas se deben liofilizar y refrigerar en todo momento durante almacenamiento, haciéndolas difíciles de transportar y/o almacenar. Los problemas de almacenamiento son particularmente agudos cuando las fórmulaciones farmacéuticas se deben almacenar y dispensar fuera del ambiente hospitalario.

Se han propuesto pocas soluciones prácticas para los problemas de almacenamiento de las moléculas proteicas lábiles. Por consiguiente, existe la necesidad de fórmulaciones de larga duración estabilizadas de moléculas terapéuticas proteináceas que sean dispensadas de manera fácil, preferiblemente con una fórmulación simple requiriendo manipulación mínima después de almacenamiento.

Ante administración *in vivo,* las proteínas terapéuticas en su estado nativo o cuando se producen de manera recombinante, tales como interferones y hormonas de crecimiento, exhiben una estabilidad plasmática corta debido a la rápida eliminación desde la corriente sanguínea. Por consiguiente, los efectos terapéuticos proporcionados por estas proteínas también son de corta duración. Así, con el objeto de sostener su efecto terapéutico deseado *in vivo*, la eliminación rápida de estas proteínas desde la sangre impone que las moléculas terapéuticas se deben administrar con mayor frecuencia o a dosis mayores. Sin embargo, aumentar el esquema de dosificación para administración de la proteína terapéutica a menudo produce un aumento en reacciones del sitio de inyección, efectos secundarios, y toxicidad en el paciente. De manera

similar, la administración de la proteína terapéutica a una dosis mayor comúnmente también produce un aumento en toxicidad y efectos secundarios en el paciente.

Las pocas soluciones practicas propuestas para aumentar la estabilidad del plasma de moléculas terapéuticas, incluyendo la conjugación química, han proporcionado beneficio limitado al paciente. Generalmente, en la mayoría de los casos, estas moléculas terapéuticas modificadas de manera química aún se administran sobre un esquema de dosificación frecuente, reteniendo reacciones de sitio de inyección, efectos secundarios, y toxicidad significativas en pacientes. Por consiguiente, existe la necesidad de una forma estabilizada de moléculas terapéuticas que retenga una mayor estabilidad del plasma in vivo que las moléculas terapéuticas naturales o producidas de manera recombinante y se pueda administrar con menor frecuencia, disminuyendo así los efectos secundarios potenciales al paciente.

RESUMEN DEL INVENTO

El presente invento se refiere a proteínas de fusión de albúmina comprendiendo una proteína Terapéutica (ej., un polipéptido, anticuerpo, o péptido, o fragmento o variante del mismo) fusionada a albúmina o un fragmento (porción) o variante de albúmina. El presente invento también contempla polinucleótidos comprendiendo, o alternativamente que consta de moléculas de ácido nucleico que codifican una proteína Terapéutica (ej., un polipéptido, anticuerpo, o péptido, o fragmento o variante de los mismos) fusionada a albúmina o una fragmento (porción) o variante de albúmina. El presente invento también incluye polinucleótidos, comprendiendo, o alternativamente constando de moléculas de ácido nucleico que codifican proteínas comprendiendo una proteína Terapéutica (ej., un polipéptido, anticuerpo, o péptido, o fragmento o variante de los mismos) fusionada a albúmina o un fragmento (porción) o variante de albúmina, que es suficiente para prolongar la vida en almacenamiento de la proteína Terapéutica, para aumentar la estabilidad del plasma de la proteína Terapéutica en comparación con su estado sin fusionar, y/o estabilizar la proteína Terapéutica y/o su actividad en solución (o en una composición farmacéutica) *in vitro* y/o *in vivo*. Las proteínas de fusión de albúmina codificadas por un polinucleótido del invento también son contempladas por el invento, como son las células huésped transformadas con polinucleótidos del invento, y métodos de fabricación de proteínas de fusión de albúmina del invento y utilización de estos polipéptidos del invento, y/o células huésped.

En un aspecto preferido del invento, las proteínas de fusión de albúmina incluyen, pero no están limitadas a aquellas descritas en la Tabla 2 y los polinucleótidos que codifican tales proteínas.

El invento también contempla fórmulaciones farmacéuticas comprendiendo una proteína de fusión de albúmina del invento y un vehículo o diluyente farmacéuticamente aceptable. Tales fórmulaciones pueden estar en un kit o recipiente. Tal kit o recipiente se puede empacar con instrucciones con respecto a la vida en almacenamiento extendida de la proteína terapéutica. Tales fórmulaciones se pueden utilizar en métodos para tratar, prevenir, aliviar o diagnosticar una enfermedad o síntoma de enfermedad en un paciente, preferiblemente un mamífero, más preferiblemente un humano, comprendiendo el paso de administrar la fórmulación farmacéutica al paciente.

En otras realizaciones, el presente invento incluye métodos para prevenir, tratar, o aliviar una enfermedad o trastorno. En realizaciones preferidas, el presente invento incluye un método para tratar una enfermedad o trastorno listado en la columna "Indicación Preferida: Y" de la tabla 1 comprendiendo administración a un paciente en el cual tal tratamiento, prevención o alivio se desea, una proteína de fusión de albúmina del invento que comprende una proteína terapéutica o

porción que comprende una proteína terapéutica (o fragmento o variante de la misma) divulgada en la columna "Proteína Terapéutica: X" de la Tabla 1 (en la misma fila que la enfermedad o trastorno a ser tratado en la columna "Indicación Preferida: Y" de la tabla 1 en una cantidad efectiva para tratar, prevenir o aliviar la enfermedad o trastorno.

En una realización, una proteína de fusión de albúmina descrita en la Tabla 1 o 2 tiene una vida en almacenamiento extendida.

En una segunda realización, una proteína de fusión de albúmina descrita en la Tabla 1 y 2 es más estable que la correspondiente molécula terapéutica no fusionada descrita en la Tabla 1.

El presente invento además incluye organismos transgénicos modificados para contener las moléculas de ácido nucleico del invento (incluyendo, pero sin limitar a, los polinucleótidos descritos en las Tablas 1 y 2), preferiblemente modificadas para expresar una proteína de fusión de albúmina.

BREVE DESCRIPCION DE LAS FIGURAS

La Figura 1A-D muestra la secuencia de aminoácidos de la forma madura de albúmina humana (SEQ ID NO:1) y un polinucleótido que la codifica (SEQ ID NO:2). Los nucleótidos 1 a 1755 de la SEQ ID NO:2 codifican la forma madura de albúmina humana 8SEQ ID NO:1).

La Figura 2 muestra el mapa de restricción del vector de clonación de pPPC0005 del depósito ATCC (*American Type Culture* Collection)PTA-3278.

La Figura 3 muestra el mapa de restricción del vector de expresión de la levadura *S. cerevisiae* pSAC35 (Sleep, BioTecnology 8:42 (1990)).

La Figura 4 muestra el efecto de varias diluciones de proteínas de fusión de albúmina de IFNb codificadas mediante ADN contenido en CID 2011 y 2053 sobre actividad SEAP en las células reporteras (ver Ejemplo 76). Las proteínas se diluyeron de manera serial de 5e-7 a 1e-14 g/ml en DMEM/FBS al 10% y se utilizaron para tratar las células reporteras ISRE-SEAP/293F. Después de 24 horas se retiraron los sobrenadantes desde las células reporteras y se sometieron a un ensayo para evaluar la actividad SEAP. La proteína de fusión de albúmina de IFNb se purificó a partir de tres clones estables: 293F/#2011, CHO/#2011 y NSO/#2053. IFNb derivado de mamífero, Avonex, provino de Biogen y se reportó con una actividad específica de 2.0e5 IU/ug.

La Figura 5 compara la actividad anti-proliferativa de la proteína de fusión de albúmina de IFN codificada mediante CID 3165 (proteína CID 3165) y IFNa recombinante (rIFNa) sobre células de melanoma Hs294T. Las células fueron cultivadas con concentraciones variables de cualquiera proteína CID 3165 o rIFNa y la proliferación se midió mediante incorporación de BrdU después de 3 días de cultivo. La proteína CID 3165 produjo inhibición medible de proliferación celular a una concentración por encima de 10 ng/ml con inhibición del 50% lograda a aproximadamente 200 ng/ml. (■)= proteína CID 3165, (♦) = rIFNa.

La Figura 6 muestra el efecto de varias diluciones de proteínas de fusión de albúmina de IFNa sobre actividad SEAP en las células reporteras ISRE-SEAP/293F. Se probó una preparación del IFNa fusionado corriente arriba de la albúmina (♦), así como también dos preparaciones diferentes de IFNa fusionado corriente abajo de albúmina (•) y (■).

La Figura 7 muestra el efecto de tiempo y dosis de proteína de fusión de albúmina de IFNa codificada mediante AND contenido en el constructo 2249 (proteína CID 2249) sobre el nivel mARN de OAS (p41) en monos tratados (ver Ejemplo 78). Punto por tiempo: primera barra = vehículo control, segunda barra = 30 ug/kg proteína CID 2249 día 1 intravenosa, tercera

barra = 30 ug/kg proteína CID 2249 día 1 subcutánea, cuarta barra = 300 ug/kg proteína CID 2249 día 1 subcutánea, quinta barra = 40 ug/kg IFNa recombinante día 1, 3 y 5 subcutánea.

La Figura 8 ilustra la relación de respuesta a dosis de proteínas de fusión de albúmina de BNP codificadas mediante ADN comprendido en los constructos CID 3691 y 3618 (proteína CID 3691 y 3618) con la activación de formación cGMP en células reporteras NPR-A/293F (ver Ejemplos 80 y 81). Tanto BNP recombinante (■), así como también dos preparaciones diferentes de BNP fusionado corriente arriba de albúmina (□) y (•) fueron probadas.

La Figura 9 muestra el efecto de la proteína de fusión de albúmina de BNP sobre presión arterial media en ratas espontáneamente hipertensas (ver Ejemplo 80). El vehículo (□), proteína BNP recombinante (•), o proteína de fusión de albúmina BNP (O) se liberaron a través de inyección de la vena de la cola. Las presiones sanguíneas sistólica y diastólica se registraron mediante el método pletismográfico en cola.

La Figura 10 muestra los niveles cGMP de plasma en ratones macho C57/BL6 de once a doce semanas de edad después de inyección intravenosa de proteína BNP recombinante (•) o proteína de fusión de albúmina BNP (O) (ver Ejemplo 80). Los niveles de cGMP se determinaron a partir de plasma preparado de sangrados de cola recolectados en varios puntos de tiempo después de inyección intravenosa.

La Figura 11 muestra los niveles de glucosa en sangre en ratones db/db diabéticos de ocho semanas de edad en ayunas 24 horas después de administración simple de fusión en tándem de GLP-1(7-36°8G)2x y HSA (albúmina sérica humana) (CID 3610) (◊), fusión monomérica de GLP-1(7-36°8G)2x y HSA (albúmina sérica humana) (◊), o HSA por si sola (•). Los niveles de glucosa en sangre se midieron mediante una prueba de tolerancia de glucosa oral. La fusión en tandem GLP-1(7-36A8G)2x-HSA (CID 3610) (◊) tuvo una actividad normalizadora de glucosa más potente en comparación con la fusión monomérica de GLP-1(7-36A8G)-HSA (Δ) 24 horas después de una administración simple en ratones db/db diabéticos de ~8 semanas de edad.

DESCRIPCION DETALLADA

Definiciones

Las siguientes definiciones se proporcionan para facilitar el entendimiento de ciertos términos utilizados en toda la especificación.

Como se utiliza en la presente, "polinucleótido" se refiere a una molécula de ácido nucleico con una secuencia nucleótida que codifica una proteína de fusión que comprende, o alternativamente consta de al menos una molécula de albúmina (o un fragmento o variante de la misma) unida en marco de lectura a por lo menos una proteína terapéutica X (o fragmento o variante de la misma); una molécula de ácido nucleico con una secuencia nucleótida que codifica una proteína de fusión comprendiendo, o alternativamente constando de, la secuencia de aminoácidos de SEQ ID NO:Y (como se describe en la columna 6 de la tabla 2) o un fragmento o variante de la misma; una molécula de ácido nucleico con una secuencia nucleótida que comprende o alternativamente consta de la secuencia mostrada en SEQ ID NO:X; una molécula de ácido nucleico con una secuencia nucleótida que codifica una proteína de fusión que comprende o alternativamente consta de la secuencia de aminoácidos de SEQ ID NO:Z; una molécula de ácido nucleico con una secuencia nucleótida que codifica una proteína de fusión de albúmina del invento generada como se describe en la Tabla 2 o en los Ejemplos; una molécula de ácido nucleico con una secuencia nucleótida que codifica una proteína de fusión de albúmina Terapéutica del invento, una molécula de ácido nucleico con una secuencia nucleótida contenida en un constructo de fusión de albúmina descrito en la Tabla 2, o una molécula de ácido nucleico

con una secuencia nucleótida contenida en un constructo de fusión de albúmina depositado con el ATCC (como se describe en la Tabla 3).

Como se utiliza en la presente, "constructo de fusión de albúmina" se refiere a una molécula de ácido nucleico que comprende o alternativamente consta de un Polinucleótido que codifica al menos una molécula de albúmina (o un fragmento o variante de la misma) unida en marco de lectura a por lo menos un polinucleótido que codifica al menos una molécula de una proteína Terapéutica (o fragmento o variante de la misma); una molécula de ácido nucleico que comprende o alternativamente consta de un polinucleótido que codifica al menos una molécula de albúmina (o un fragmento o variante de la misma) unida en marco de lectura a por lo menos un Polinucleótido que codifica al menos una molécula de una proteína Terapéutica (o fragmento o variante de la misma) generada como se describe en la Tabla 2 o en los Ejemplos; o una molécula de ácido nucleico que comprende o alternativamente consta de un Polinucleótido que codifica al menos una molécula de albúmina (o un fragmento o variante de la misma) unida en marco de lectura a por lo menos un Polinucleótido que codifica al menos una molécula de una proteína Terapéutica (o fragmento o variante de la misma), además comprendiendo por ejemplo, uno o más de los siguientes elementos (1) un vector de auto-replicación funcional (incluyendo pero sin limitar a un vector de lanzadera, un vector de expresión, un vector de integración, y/o un sistema de replicación), (2) una región para iniciación de trascripción (ej., una región promotora, tal como por ejemplo, un promotor regulable o inducible, un promotor constitutivo, (3) una región para terminación de trascripción, (4) una secuencia lider, y (5) un marcador seleccionable. El Polinucleótido que codifica la proteína Terapéutica y proteína de albúmina, una vez parte del constructo de fusión de albúmina, puede cada uno denominarse como "porción", "región" o "fracción" del constructo de fusión de albúmina.

Generalmente, el presente invento se relaciona con polinucleótidos que codifican proteínas de fusión de albúmina; proteínas de fusión de albúmina; y métodos para tratar, prevenir, o aliviar enfermedades o trastornos utilizando proteínas de fusión de albúmina o polinucleótidos que codifican proteínas de fusión de albúmina. Como se utiliza en la presente, "proteína de fusión de albúmina" se refiere a una proteína formada por la fusión de al menos una molécula de albúmina (o un fragmento o variante de la misma) a al menos una molécula de una proteína Terapéutica (o fragmento o variante de la misma). Una proteína de fusión de albúmina del invento comprende al menos un fragmento o variante de una proteína Terapéutica y al menos un fragmento o variante de albúmina sérica humana, las cuales están asociadas entre sí mediante fusión genética (es decir, la proteína de fusión de albúmina es generada mediante traslación de un ácido nucleico en el cual un Polinucleótido que codifica toda o una porción de una proteína Terapéutica está unida en marco de lectura con un Polinucleótido que codifica toda o una porción de albúmina). La proteína terapéutica y proteína de albúmina, una vez parte de la proteína de fusión de albúmina, cada una se puede denominar "porción", "región", o "fracción" de la proteína de fusión de albúmina (ej., una "porción de proteína terapéutica" o una "porción de proteína de albúmina"). En una realización altamente preferida, una proteína de fusión de albúmina del invento comprende al menos una molécula de una proteína Terapéutica X o fragmento o variante de la misma (incluyendo, pero sin limitar a una forma madura de la proteína terapéutica X) y al menos una molécula de albúmina o fragmento o variante de la misma (incluyendo pero sin limitar a una forma madura de albúmina).

En una realización adicional preferida, una proteína de fusión de albúmina del invento es procesada mediante una célula huésped y secretada en el medio de cultivo circundante. El procesamiento de la proteína de fusión de albúmina naciente que ocurre en las rutas secretorias del huésped utilizado para expresión puede incluir, pero no se limita a escisión del péptido de

señal; formación de enlaces de disulfuro; doblamiento apropiado; adición y procesamiento de carbohidratos (tal como por ejemplo, glicosilación N- y O-ligada); escisiones proteolíticas específicas; y ensamble en proteínas multiméricas. Una proteína de fusión de albúmina del invento preferiblemente está en la forma procesada. En una realización más preferida, la "forma procesada de una proteína de fusión de albúmina" se refiere a un producto de proteína de fusión de albúmina que se haya sometido a una escisión del péptido de señal por el extremo N-terminal, conocido también en la presente como "proteína de fusión de albúmina madura".

En varias instancias, un clon representativo que contiene un constructo de fusión de albúmina del invento fue depositado con la Colección de Cultivo del Tipo Americano (conocida en la presente como "ATCC®"). Además, es posible recuperar un constructo de fusión de albúmina dado desde el depósito mediante técnicas conocidas en el estado de la técnica y descritas en la presente. El ATCC® está localizado en 10801 Univertsity Boulevard, Manassas, Virginia 20110-2209, USA. Los depósitos ATCC® fueron hechos conforme a los términos del Tratado de Budapest sobre el reconocimiento internacional del depósito de microorganismos para los propósitos del procedimiento de patentes.

En una realización, el invento proporciona un Polinucleótido que codifica una proteína de fusión de albúmina que comprende o alternativamente consta de una proteína Terapéutica y una proteína de albúmina sérica. En una realización adicional, el invento proporciona una proteína de fusión de albúmina que comprende, o alternativamente consta de una proteína Terapéutica y una proteína de albúmina sérica. En una realización preferida, el invento proporciona una proteína de fusión de albúmina que comprende o alternativamente consta de una proteína Terapéutica y una proteína de albúmina sérica codificada mediante un polinucleótido descrito en la Tabla 2. En una realización adicionalmente preferida, el invento proporciona un Polinucleótido que codifica una proteína de fusión de albúmina cuya secuencia se muestra como SEQ ID NO:Y en la Tabla 2. En otras realizaciones, el invento proporciona una proteína de fusión de albúmina que comprende o alternativamente consta de un fragmento biológicamente activo y/o terapéuticamente activo de una proteína Terapéutica y una proteína de albúmina sérica. En otras realizaciones, el invento proporciona una proteína de fusión de albúmina que comprende o alternativamente consta de una variante biológicamente activa y/o terapéuticamente activa de una proteína Terapéutica y una proteína de albúmina sérica. En realizaciones preferidas, el componente de proteína de albúmina sérica de la proteína de fusión de albúmina es la porción madura de la albúmina sérica. El invento además incluye polinucleótidos que codifican estas proteínas de fusión de albúmina.

En realizaciones adicionales, el invento proporciona una proteína de fusión de albúmina que comprende o alternativamente consta de una proteína Terapéutica, y un fragmento biológicamente activo y/o terapéuticamente activo de albúmina sérica. En realizaciones adicionales, el invento proporciona una proteína de fusión de albúmina que comprende o alternativamente consta de una proteína Terapéutica y una variante biológicamente activa y/o terapéuticamente activa de albúmina sérica. En realizaciones preferidas, la porción de proteína Terapéutica de la proteína de fusión de albúmina es la porción madura de la proteína Terapéutica. En una realización adicionalmente preferida, la porción de proteína Terapéutica de la proteína de fusión de albúmina es el dominio soluble extracelular de la proteína Terapéutica. En una realización alternativa, la porción de proteína Terapéutica de la proteína de fusión de albúmina es la forma activa de la proteína Terapéutica. El invento además incluye polinucleótidos que codifican estas proteínas de fusión de albúmina.

En realizaciones adicionales, el invento proporciona una proteína de fusión de albúmina que comprende o alternativamente consta de una variante o fragmento biológicamente activo y/o

terapéuticamente activo de una proteína terapéutica y/o fragmento o variante biológicamente activo y/o terapéuticamente activo de albúmina sérica. En realizaciones preferidas, el invento proporciona una proteína de fusión de albúmina que comprende o alternativamente consta de la porción madura de una proteína Terapéutica y la porción madura de albúmina sérica. El invento además incluye polinucleótidos que codifican estas proteínas de fusión de albúmina.

Proteínas Terapéuticas

Como se afirmó anteriormente, un Polinucleótido del invento codifica una proteína que comprende o alternativamente consta de al menos un fragmento o variante de una proteína Terapéutica y al menos un fragmento o variante de albúmina sérica humana, las cuales están asociadas entre si, preferiblemente mediante fusión genética.

Una realización adicional incluye un Polinucleótido que codifica una proteína que comprende o alternativamente consta de al menos un fragmento o variante de una proteína Terapéutica y al menos un fragmento o variante de albúmina sérica humana, los cuales están unidos entre si mediante conjugación química.

Como se utiliza en la presente, "proteína Terapéutica" se refiere a proteínas, polipéptidos, anticuerpos, péptidos o fragmentos o variantes de los mismos, que tienen una o más actividades terapéuticas y/o biológicas. Las proteínas terapéuticas contempladas por el invento incluyen pero no están limitadas a proteínas, polipéptidos, péptidos, anticuerpos, y biológicos. (Los términos péptidos, proteínas, y polipéptidos se utilizan de manera intercambiable en la presente.) Específicamente se contempla que el término "Proteína Terapéutica" contempla anticuerpos y fragmentos y variantes de los mismos. Así una proteína del invento pueden contener al menos un fragmento o variante de una proteína Terapéutica, y/o al menos un fragmento o variante de un anticuerpo. Adicionalmente, el término "Proteína Terapéutica" puede referirse al correlato endógeno o de ocurrencia natural de una proteína Terapéutica.

Polipéptido que presenta una "actividad terapéutica" o una proteína que es "terapéuticamente activa" se refiere a un polipéptido que posee una o más actividades biológicas y/o terapéuticas conocidas asociadas con una proteína terapéutica tal como una o más proteínas Terapéuticas descritas en la presente o de otra forma conocidas en el estado de la técnica. Como ejemplo no limitante, una "proteína Terapéutica" es una proteína útil para tratar, prevenir o aliviar una enfermedad, condición o trastorno. Como ejemplo no limitante, una "proteína Terapéutica" puede ser aquella que se fija específicamente a un tipo de célula particular (normal (e., linfocitos) o anormal ej., (células cancerigenas)) y por lo tanto se puede utilizar para dirigir un compuesto (fármaco o agente citotóxico) a ese tipo de célula específicamente.

Por ejemplo, una lista no exhaustiva de porciones de "proteínas Terapéuticas" las cuales pueden estar compuestas por una proteína de fusión de albúmina del invento incluye, pero no está limitada a GLP-1, GLP-2, PACAP-27, PACAP-28, VIP, CD4M33, secretina, glicentina, oxintomodulina, PHM, IFNα, IFNβ, ANP, BNP, NGF, BDNF, GDNF, y somatostatina.

También se pueden fusionar híbridos de interferón al extremo amino o carboxilo de albúmina para formar una proteína de fusión de albúmina e híbridos de interferón. La proteína de fusión de albúmina e híbridos de interferón pueden tener actividad de interferón aumentada, o alternativamente suprimida, tales como respuestas antivirales, regulación de crecimiento celular, y modulación de respuesta inmune (Lebleu, *PNAS* USA, 73:3107-3111 (1976); Gresser, *Nature*, 251:543-545 (1974); y Jonson, *Texas Reports Biol Med*, 35:357-369 (1977)). Cada proteína de fusión de albúmina e híbridos de interferón se pueden utilizar para tratar, prevenir, o aliviar infecciones virales (ej., hepatitis (ej., VHC); o VIH), esclerosis múltiple, o cáncer.

En una realización, la porción de hibrido de interferón de la proteína de fusión de albúmina de híbrido de interferón comprende un hibrido de interferón alfa- interferón alfa (citado en la presente como híbrido alfa-alfa). Por ejemplo, la porción de híbrido alfa-alfa de la proteína de fusión de albúmina de híbrido de interferón consta o alternativamente comprende interferón A fusionado a interferón alfa D. En una realización adicional, el híbrido A/D está fusionado en el sitio de restricción BglII común a interferón alfa D, en donde la porción N-terminal del híbrido A/D corresponde a los aminoácidos 1-62 de interferón alfa A y la porción C-terminal corresponde a los aminoácidos 64-166 de interferón alfa D. Por ejemplo, este híbrido A/D comprendería la secuencia:

CDLPQTHSLGSRRTLMLLAQMRX1ISLFSCLKDRHDFGFQEEFGNQFQKAETIPVL HEMIQQIFNLFTTKDSSAAWDEDLLDFCTELYQQLNDLEACVMQEERVGETPLMNX2DS ILAVKKYFRRITLYLTEKKYSPCAWEVVRAEIMRSLSLSTNLQERLRRKE (SEQ ID NO.99), en donde el X_1 es R o K y el X_2 es A o V (ver, por ejemplo, Constructo ID #2875). En una realización adicional, el híbrido A/D está fusionado en el sitio de restricción común PvuIII, en donde la porción N-terminal del hibrido A/D corresponde los animoácidos 1-91 de interferón alfa A y la porción C terminal corresponde a los aminoácidos 93-166 de interferón alfa D. Por ejemplo este híbrido A/D comprendería la secuencia de aminoácidos:
CDLPQTHSLGSRRTLMLLAQMRX1ISLFSCLKDRHDFGFPOQEEFGNQFQKAETIPVLHE MI9QQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVMQEERVGETPLMNX2DSI LAVKKYFRRITLYLTEKKYSPCAWEVVRAEIMRSLSLSTNLQERLRRKE (SEQ ID NO.100), en donde el X_1 es R o K y el segundo X_2 es A o V (ver, por ejemplo, Constructo ID #2872). Estos híbridos adicionalmente se describen en la Patente Americana No. 4.414.510, la cual se incorpora en la presente como referencia en su totalidad.

En una realización adicional, la porción de híbrido alfa-alfa de la proteína de fusión de albúmina de híbrido de interferón consta o alternativamente comprende interferón alfa A fusionada a interferón alfa F. En una realización adicional, el hibrido A/F es fusionado en el sitio de restricción común PvuIII, en donde la porción N-terminal del hibrido A/F corresponde a los aminoácidos 1-91 de interferón alfa A y la porción c-terminal corresponde a los aminoácidos 93-166 de interferón alfa F. Por ejemplo, este híbrido A/F comprendería la secuencia de aminoácidos:
CDLPQTHSLGSRRTLMLLAQMRXISLFSCLKDRHDFGFQEEFGNQFQKAETIPVLHEMIQ QIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDMEACVIQEVGVEETPLMNVDSILAVK KFQRITLYLTEKKYSPCAWEVVRAEIMRSFSLSKIFQERLRRKE (SEQ. ID NO:101), en donde X es uno u otro R o K (ver, por ejemplo, Constructo ID #2874). Estos híbridos además se describen en la Patente Americana No. 4.414.510, la cual es incorporada en la presente como referencia en su totalidad. En una realización adicional, la porción de hibrido alfa-alfa de la proteína de fusión de albúmina de híbrido de interferón consta o alternativamente comprende interferón alfa A fusionada a interferón alfa B. En una realización adicional, el híbrido A/B está fusionado en el sitio de restricción común PvuIII, en donde la porción N-terminal del híbrido A/B corresponde a los aminoácidos 1-91 de interferón alfa A y la porción C-terminal corresponde a los aminoácidos 93-166 de interferón alfa B. Por ejemplo, este hibrido A/B comprendería la secuencia de aminoácidos:
CDLPQTHSLGSRRTLMLLAQMRXISLFSCLKDRHDFGFQEEFGNQFQKAETIPVLHEMIQ QIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEX2X3X4X5QEVGVIESPLMYEDSILA VRKYFQRITLYLTEKKYSSCAWEVVRAEIMRSFSLSINLQKRLKSKE (SEQ. ID NO:102), en donde X_1 es R o K y X_2 a X_5 es SCVM o VLCD (ver, por ejemplo, Constructo ID #2873).

Estos híbridos se describen adicionalmente en la Patente Americana No. 4.414.510, la cual se incorpora en la presente como referencia en su totalidad.

En otra realización, la porción de hibrido de interferón de la proteína de fusión de albúmina de híbrido de interferón comprende un hibrido de interferón beta-interferón alfa (citado en la presente como híbrido beta-alfa). Por ejemplo, la porción de hibrido beta-alfa de la proteína de fusión de albúmina de híbrido de interferón consta o alternativamente comprende interferón beta-1 fusionada a interferón alfa D (también conocida como interferón alfa-1). En una realización adicional, el hibrido beta-1/alfa D está fusionado en donde la porción N-terminal corresponde a los aminoácidos 1-73 de interferón beta-1 y la porción C-terminal corresponde a los aminoácidos 74-167 de interferón alfa D. Por ejemplo, este hibrido beta-1/alfa D comprendería una secuencia de aminoácidos:
MSYNLLGFLQRSSNFQCQKLLWQLNGRLRYCLKDRMNFDIPEEIKQLQQFQKEDAALTI YEMLQNIFAIFQDSSAAWDEDLLDKFCTELYQQLNDLEACVMQEERVGETPLMNXDSIL AVKKYFRRITLYLTEKKYSPCAWEVVRAEIMSLSLSTNLQERLRRKE (SEQ. ID NO:103), en donde X es A o V. Estos híbridos se describen adicionalmente ente la Patente Americana No. 4.758.428, la cual se incorpora en la presente como referencia en su totalidad.

En otra realización, porción de hibrido de interferón de la proteína de fusión de albúmina de híbrido de interferón comprende un hibrido de interferón alfa-interferón beta (citado en la presente como híbrido alfa-beta). Por ejemplo, la porción de hibrido alfa-beta de la proteína de fusión de albúmina de híbrido de interferón consta o alternativamente comprende interferón alfa D (también citada como interferón alfa-1) fusionada a interferón beta-1. En una realización adicional, el hibrido D/beta-1 es fusionado en donde la porción N-terminal corresponde a los aminoácidos 1-73 de interferón alfa D y la porción C-terminal corresponde a los aminoácidos 74-166 de interferón beta-1. Por ejemplo, este hibrido alfa D/beta-1 tendría una secuencia de aminoácidos:
MCDLPETHSLDNRRTLMLLAQMSRISPSSCLMDRHDFGFPQEEFDGNQFQKAPAISVLHE LIQQIFNLFTTKDSSSTGWNETIVELLANVYHQINHLKTVLEEKLEKEDFTRGKLMSSLHL KRYYGRILHYLKAKEYSHCAWTIVRVEILRNFYFINRLTGYLRN (SEQ. ID NO:104). Estos híbridos se describen adicionalmente en la Patente Americana No. 4.758.428 la cual se incorpora en la presente como referencia en su totalidad.

En realizaciones adicionales, la porción de hibrido de interferón de las proteínas de fusión de albúmina de híbrido de interferón pueden comprender combinaciones adicionales de híbridos de interferón alfa-alfa, híbridos de interferón alfa-beta e híbridos de interferón beta-alfa. En realizaciones adicionales, la porción de hibrido de interferón de la proteína de fusión de albúmina de híbrido de interferón se puede modificar para incluir mutaciones, sustituciones, eliminaciones, o adiciones a la secuencia de aminoácidos del híbrido de interferón. Tales modificaciones a las proteínas de fusión de albúmina de híbrido de interferón se pueden realizar, por ejemplo, para mejorar los niveles de producción, aumentar estabilidad, aumentar o disminuir actividad, o conferir nuevas propiedades biológicas.

Las proteínas de fusión de albúmina de híbridos de interferón anteriormente descritas son contempladas por el invento, como son células huésped y vectores que contienen polinucleótidos que codifican los polipéptidos. En una realización, una proteína de fusión de albúmina de híbrido de interferón codificada por un polinucleótido como se describió anteriormente, tiene una vida en almacenamiento extendida. En una realización adicional, una proteína de fusión de albúmina de híbrido de interferón codificada por un polinucleótido descrito anteriormente tiene una vida media sérica y/o actividad más estabilizada en solución (o en una composición farmacéutica) *in*

vivo y/o *in vitro* en comparación con la correspondiente molécula de híbrido de interferón no fusionada.

En otro ejemplo no limitante, una "proteína Terapéutica" es una proteína que tiene actividad biológica, y en particular, una actividad biológica útil para tratar, prevenir o aliviar una enfermedad. Una lista no inclusiva de actividades biológicas que una proteína Terapéutica puede poseer incluye, inhibición de infección VIH-1 de células, estimulación de proliferación celular epitelial intestinal, reducción de permeabilidad celular epitelial intestinal, estimulación de secreción de insulina, inducción de broncodilatación y vasodilatación, inhibicación de aldosterona y secreción de renina, regulación de presión sanguínea, promoción de crecimiento neuronal, aumento de respuesta inmune, aumento de inflamación, eliminación de apetito, o cualquiera o más actividades biológicas descritas en la sección "Actividades Biológicas" y/o divulgadas para una proteína Terapéutica dada en la Tabla 1 (columna 2).

En una realización, las fusiones de IFN-beta y albúmina sérica humana (HSA) se utilizan para inhibir la actividad del virus Ebola y el virus del SARS (Síndrome agudo respiratorio severo) (cepa Toronto-2). Por ejemplo, la actividad antiviral in vitro de IFN-beta fusionada corriente arriba de HSA madura (proteína CID 2053) se evaluó contra el virus Ebola y virus SARS en células Vero. Estas células se utilizaron para evaluar los efectos protectores de la proteína CID 2053 con base en inhibición de efecto citopático (CPE) y el ensayo de rojo neutro de viabilidad celular. La transducción de señales fue evaluada in vitro mediante análisis de expresión genética. Además, la farmacocinética y farmacodinámica de la proteína CID 2053 fueron evaluadas en monos rhesus. Los resultados indican que la actividad antiviral in Vitro potente fue lograda con un índice de seguridad favorable. El IC50 para la proteína CID 2053 fue 0.4 ng/ml contra Ebola y 2 ng/ml contra el virus SARS. El análisis por microarreglos mostró que la proteína CID 2053 y IFN-beta inducen la expresión de un grupo similar de genes y activan la ruta de transducción de señales de elemento de respuesta IFN-estimulada (ISRE). En monos rhesus se administró una dosis de 50 ug/kg iv (intravenoso) o SC (subcutáneo) o 300 uG/kg SC proteína 2053 CID, la vida media terminal fue 36-40 horas. La administración de proteína CID 2053 indujo aumentos sostenidos en niveles de neopterina sérica y expresión mARN OAS1.

En una realización adicional, las fusiones de IFN-alfa-HSA se utilizan para inhibir agentes virales clasificados bajo la categoría A. Filo (Ebola), Arena (Pichende), Categoría B- Toga (VEE) o Categoría C- Bunya (Punto toro), Flavi (fiebre Amarilla, Nilo Occidental). Por ejemplo, se utilizaron ensayos de inhibición del CPE (efecto citopático), teñido con rojo neutro y ensayos de rendimiento viral para evaluar las actividades antivirales de INF-alfa fusionado corriente abajo de HSA (proteína CID 3165). Se evaluó la actividad farmacocinética y farmacodinámica de la proteína CID 3165 en monos cinomolgus y sujetos humanos. Los resultados indican que la actividad antiviral se logró contra todos los virus de ARN evaluados con un índice de seguridad favorable. Los valores IC50 oscilaron entre 0.1 ng/ml (Punta Toro A) y 19 ng/ml (VEE) en el ensayo CPE. En monos cynomolgus, la vida media de la proteína CID 3165 fue 90 horas y fue detectable hasta 14 días después de la dosis. En sujetos humanos, la proteína CID 3165 fue segura y bien tolerada. C_{max} después de dosis de inyección simples fue proporcional a dosis. El C_{max} medio en el grupo de 500 ug fue 22 ng/ml, y el $t_{1/2}$ medio fue 150 horas. La dosificación una vez cada 2-4 semanas o más es soportada por la farmacocinética. Se observó respuesta antiviral contra hepatitis C en la mayoria de sujetos en los grupos de inyección única (120-500 ug).

En una realización adicional, las fusiones IFN-alfa-HSA se utilizan para tratar pacientes con infección de hepatitis C crónica (VHC). La interferón alfa, también conocida como interferón alfa o interferón leucocito, es el estándar terapéutico para pacientes infectados con VHC (virus de

la hepatitis C). El término "interferón alfa" se refiere a una familia de polipéptidos relacionados altamente homólogos con actividad antiviral. La porción de interferón alfa de la fusión IFN-alfa-HSA consta o alternativamente comprende cualquier interferón alfa o fragmento de la misma conocida en el estado de la técnica. Ejemplos no limitantes de interferón alfa contemplados por el invento incluyen, pero no están limitados a las proteínas de interferón alfa divulgadas en la columna de proteínas Terapéuticas de la Tabla 1. En realizaciones particulares, la porción de interferón alfa consta o alternativamente comprende interferón alfa-2a, interferón alfa-2b, interferón alfa-2c, interferón de consenso, interferón alfacon-1, interferón alfa-n1, interferón alfa-n3, cualquier forma comercialmente disponible de interferón alfa, tal como, por ejemplo INTRON® A (Schering Corp., Kenilworth, N.J.), ROFERON® A (Hoffman-La Roche, Nutley, N.J.), interferón alfa Berofor (Boehringer Ingelheim Pharmaceutical, Inc., Ridgefied, Conn.), OMNIFERON™ (Viragen, Inc., Plantation, FL), MULTIFERON™ (Viragen, Inc., Plantation, FL) WELLFERON® (GlaxoSmithKline, Londres, Gran Bretaña), INFERGEN® (Amgen, Inc, Thousands Oaks, CA), SUMIFERON® (Sumitomo, Japón), BELEROFON ® (Nautilus Biotech, Francia) o cualquier producto interferón alfa purificado o un fragmento del mismo. En realizaciones adicionales, la porción interferón alfa de la proteína de fusión IFN-alfa-HSA se puede modificar mediante la unión de fracciones químicas. Por ejemplo, la porción de interferón alfa puede ser modificada mediante pegilación. Por consiguiente, en realizaciones adicionales, la porción de interferón alfa de la proteína de fusión IFN-alfa-HSA consta o alternativamente comprende formas pegiladas de interferón alfa-2a, 2b, o interferón de consenso e incluyen, pero no están limitadas a interferón alfa pegilada comercialmente disponible, tal como, por ejemplo, PEG-INTRON® (Schering Corp., Kinilworth, N.J.), PEGASYS® (Hoffman-La Roche, Nutley, N.J.), PEG-OMNIFERON™ ((Viragen, Inc., Plantation, FL)) o un fragmento del mismo. Sin embargo, como se utiliza en la presente, fusiones "IFN-alfa-HSA" se refiere a HSA fusionada a cualquiera de las proteínas interferón alfa conocidas en el estado de la técnica o un fragmento de las mismas.

Los pacientes infectados con VHC pueden estar dentro de dos categorías con base en exposición previa a un regimen de interferón para tratamiento de la infección VHC. "Pacientes ingenuos" son aquellos a quienes nunca se ha tratado con un regimen de interferón. "Pacientes experimentados" son aquellos pacientes quienes han sido tratados o actualmente están siendo tratados con un régimen de interferón. "No respondientes" son pacientes experimentados que han sido tratados previamente con un régimen de interferón pero han fallado para cumplir el punto final primario de tratamiento tal como reducción de carga viral temprana (EVR) o una respuesta de final de tratamiento (ETR). Sin embargo, como se utiliza en la presente, un "paciente VHC" se refiere a un paciente infectado con VHC y quien puede ser ingenuo, experimentado o no respondiente.

Además, el virus de la Hepatitis C se puede clasificar en cuatro genotipos, genotipo 1, 2, 3, o 4. Generalmente, el virus de la Hepatitis C que infecta un paciente VHC comprende un único genotipo. Sin embargo, el virus de la Hepatitis B puede comprender una combinación de dos o más genotipos. Además, el genotipo del virus de la Hepatitis C también puede ser una variante de uno de los genotipos VHC conocidos. En un realización adicional, el virus de la Hepatitis C del paciente VHC es el genotipo 1 o una variante del mismo. Sin embargo, como se utiliza en la presente, "VHC" se refiere al virus de la Hepatitis C de cualquier genotipo, o combinación o variantes del mismo.

El régimen de tratamiento estándar con VHC involucra tratamiento con interferón alfa en combinación con un agente antiviral, tal como, ribavirina. En general, la interferón alfa es

administrada diariamente, dos veces por semana, o semanalmente y la ribavirina es administrada diariamente. Sin embargo estudios recientes también han utilizado interferón alfa en combinación con otros agentes antivirales conocidos en el estado de la técnica para tratamiento de VHC. Por lo tanto en una realización adicional la fusión de IFN-alfa-HSA se puede administrar al paciente VHC sola o en combinación con un agente antiviral, tal como, por ejemplo, ribavirina.

Como se anotó anteriormente, la farmacocinética de la proteína CID 3165 soporta un esquema de dosificación de una vez cada 2-4 semanas o más. Por lo tanto, en una realización adicional, los pacientes VHC son tratados con una fusión IFN-alfa-HSA mediante administración una vez cada 2-4 semanas sola o en combinación con una cantidad efectiva de un agente antiviral. En una realización preferida, los pacientes VHC son tratados con una fusión IFN-alfa-HSA mediante administración una vez cada 2-4 semanas en combinación con una cantidad efectiva de un agente antiviral. En una realización preferida adicional, la fusión IFN-alfa-HSA se administra al paciente VHC cada cuatro semanas. En una realización preferida adicional, la fusión IFN-alfa-HSA se administra al paciente VHC más de una vez cada 4 semanas. En realizaciones adicionales, la fusión IFN-alfa-HSA es administrada una vez cada cuatro semanas o más a un paciente VHC, en donde el tratamiento también incluye administración de una cantidad efectiva de un agente antiviral.

En otra realización, las fusiones IFN-alfa-HSA se pueden utilizar como una monoterapia de dosis bajas para terapia de mantenimiento de VHC. En una realización adicional, las fusiones IFN-alfa-HSA se pueden utilizar en combinación con ribavirina y uno o más agentes antivirales para el tratamiento de VHC. Alternativamente, en otra realización, las fusiones IFN-alfa-HSA se pueden utilizar en combinación con uno o más agentes antivirales, diferentes de ribavirina, para el tratamiento de VHC. En una realización adicional, las fusiones IFN-alfa-HSA se pueden utilizar para el tratamiento de otras infecciones virales. Por ejemplo, en una realización, las fusiones IFN-alfa-HSA se pueden utilizar para el tratamiento de Hepatitis B (HBV). En una realización adicional, las fusiones IFN-alfa-HSA se pueden utilizar para el tratamiento del virus del Papiloma Humano (HPV). En una realización adicional, las fusiones IFN-alfa-HSA se pueden utilizar en el tratamiento de cáncer, incluyendo, pero sin limitar a leucemia de células vellosas, melanoma maligno, linfoma folicular, leucemia mielógena crónica, Sarcoma de Kaposi relacionado con SIDA, mieloma múltiple, o cáncer de células renales.

En otra realización, las fusiones GLP-1 HSA se utilizan para regular niveles de glucosa en sangre de pacientes diabéticos. En una realización adicional, las fusiones en tandem de GLP-1 mutante o tipo silvestre se utilizan para regular niveles de glucosa en sangre en pacientes diabéticos. Por ejemplo, se evaluó la capacidad de las fusiones GLP-1(7-36ª8G)-HSA) monómera y GLP-1(7-36ª8G)-HSA (CID) 3610) en tandem para regular los niveles de glucosa en sangre utilizando una prueba de tolerancia de glucosa (1 gram glucosa/kg cebadura oral), después de inyección subcutánea de proteína GLP-1-HSA en ratones db/db diabéticos de ~8 semanas de edad. Esta prueba de tolerancia de glucosa consistió en la inyección subcutánea de una fusión GLP-1-HSA seguida por administración de 1 gramo de glucosa/kg de cebadura oral. A ratones db/db diabéticos en ayunas se administraron dosis equimolares (100 y 171 nmol/kg) de una u otra proteína de fusión GLP-1-HSA en tandem o de nonómero y se realizaron pruebas de tolerancia de glucosa 6 o 24 horas después de administración única. De manera sorprendente e inesperada, la fusión GLP-1(7-36A8G)-HSA en tandem (CID 3610) redujo significativamente la glucosa en sangre a 6 horas después de inyección en comparación con la fusión GLP-1(7-36A8G)-HSA de monómero. Además, la diferencia entre las fusiones GLP-1(7-36A8G)-HSA de monómero y GLP-1(7-36A8G)-HSA (CID 3610) en tandem fueron aún más dramáticas cuando los ratones db/db diabéticos fueron evaluados 24 horas después de la inyección. Como se muestra en la

Figura 11, la fusión GLP-1(7-36A8G)-HSA (CID 3610) (◊) tuvo una actividad normalizadota de glucosa inesperadamente potente mientras los niveles de glucosa en sangre en ayuno para la fusión GLP-1(7-36A8G)-HSA (Δ) fueron similares para animales a los que se administró solo HSA (•) y claramente fueron de naturaleza diabética.

Como se utiliza en la presente, "actividad terapéutica" o "actividad" se puede referir a una actividad cuyo efecto es consistente con un resultado terapéutico deseable en humanos, o a efectos deseados en mamíferos no humanos o en otras especies u organismos. La actividad terapéutica se puede medir *in vivo* o *in vitro*. Por ejemplo, se puede realizar un ensayo para evaluar un efecto deseable en un cultivo celular. Tales ensayos *in Vitro* o de cultivos celulares son comúnmente disponibles para muchas proteínas terapéuticas como se describió en el estado de la técnica. Ejemplos de ensayos incluyen, pero no están limitados a aquellos descritos en la presente en la sección de Ejemplos o en la columna "Ensayo de Actividad de Ejemplo" (columna 3 de Tabla 1.

Las proteínas Terapéuticas correspondientes a una porción de proteína Terapéutica de una proteína de fusión de albúmina del invento, tales como proteínas se superficie celular y secretorias, a menudo son modificadas mediante la unión de uno o más grupos oligosacáridos. La modificación, citada como glicosilación, puede afectar dramáticamente las propiedades físicas de proteínas y puede ser importante en estabilidad, secreción, y localización de proteínas. La glicosilación ocurre en locaciones específicas a lo largo del esqueleto del polipéptido. Usualmente existen dos tipos principales de glicosilación: glicosilación caracterizada por oligosacáridos O-ligados, los cuales están fijados a residuos de serina o treonina; y glicosilación caracterizada por oligosacáridos N-ligados, los cuales son fijados a residuos de asparagina en una secuencia Asn-X-Ser o Asn-X-Thr, donde X puede ser un aminoácido excepto prolina. El ácido N-acetilneurámico (también conocido como ácido siálico) usualmente es el residuo terminal tanto de oligosacáridos N-ligados como O-ligados. Variables tales como estructura de proteínas y tipo de células influencian el número y la naturaleza de las unidades de carbohidrato dentro de las cadenas en diferentes sitios de glicosilación. Los isomeros de glicosilación también son comunes en el mismo sitio dentro de un tipo de células dado.

Las proteínas terapéuticas correspondientes a una porción de proteína Terapéutica de una proteína de fusión de albúmina del invento, así como también analogos y variantes de las mismas, se pueden modificar de manera que la glicosilación en uno o más sitios es alterada como resultado de manipulación de sus secuencias de ácidos nucleicos, mediante la célula huésped en la cual son expresadas, o debido a otras condiciones de su expresión. Por ejemplo los isomeros de glicosilación se pueden producir eliminando o introduciendo sitios de glicosilación, *ej.*, mediante sustitución o eliminación de residuos aminoácidos, tal como sustitución de glutamina por asparagina, o las proteínas recombinantes no glicosiladas se pueden producir al expresar las proteínas en células huésped que no las glicolizarán, ej., en *E. coli* o levadura deficiente de glicosilación. Estos enfoques se describen en detalle a continuación y son conocidos en el estado de la técnica.

Las proteínas terapéuticas, particularmente aquellas descritas en la Tabla 1, y sus secuencias de ácidos nucleicos y aminoácidos son bien conocidas en el estado de la técnica y están disponibles en bases de datos públicas tales como Bases de Datos de Servicios de Resúmenes Químicos (ej., el Registro CAS), GenBank, y bases de datos proporcionadas por suscripción tales como GenSeq (ej., Derwent). Secuencias nucleótidas de ejemplo de proteínas Terapéuticas las cuales se pueden utilizar para derivar un polinucleótido del invento se muestran en la columna 7, "SEQ ID NO:X," de la Tabla 2. Las secuencias mostradas en SEQ ID NO:X pueden ser una secuencia de polinucleótidos de tipo silvestre que codifica una proteína

Terapéutica dada (ej., ya sea de longitud completa o madura), o en algunas instancias la secuencia puede ser una variante de dicha secuencia polinucleotida de tipo silvestre (ej., un Polinucleótido que codifica la proteína Terapéutica de tipo silvestre, en donde la secuencia de ADN de dicho Polinucleótido se ha optimizado, por ejemplo, para expresión en una especie particular; o un Polinucleótido que codifica una variante de la proteína Terapéutica de tipo silvestre (es decir, un mutante dirigido a sitio; una variante alélica)). Está dentro de la capacidad de alguien con experiencia en el estado de la técnica, utilizar la secuencia mostrada como SEQ ID NO:X para derivar el constructo descrito en la misma fila. Por ejemplo, si la secuencia SEQ ID NO:X corresponde a una proteína de longitud completa, pero solamente una porción de esa proteína se utilizar para generar el CID específico, está dentro de la destreza del estado de la técnica confiar en técnicas de biología molecular, tales como PCR, para amplificar el fragmento específico y clonarlo en el vector apropiado.

Proteínas terapeuticaas adicionales que corresponden a una porción de proteína terapéutica de una proteína de fusión de albúmina del invento incluyen, pero no están limitadas a, una o más proteínas Terapéuticas o péptidos divulgados en la columna "Proteína Terapéutica X" de la Tabla 1 (columna 1), o fragmento o variante de las mismas.

La Tabla 1 proporciona una lista no exhaustiva de proteínas Terapéuticas que corresponden a una porción de proteína terapéutica de una proteína de fusión de albúmina del invento, o una proteína de fusión de albúmina codificada por un Polinucleótido del invento. La primera columna, "Proteína Terapéutica X," divulga moléculas de proteínas Terapéuticas que pueden ser seguidas por paréntesis conteniendo nombres cientificos o comerciales de proteínas que comprenden, o alternativamente constan de esa molécula de proteína Terapéutica o un fragmento o variante de la misma. "Proteína Terapéutica X" como se utiliza en la presente puede referirse ya sea a una molécula de proteína terapéutica, o a todo el grupo de proteínas terapéuticas asociadas con una molécula de proteína terapéutica dada en esta columna. La columna "actividad biologica" (columna 2) describe actividades biológicas asociadas con la molécula de proteínas terapéuticas. La columna 3, "Ensayo de Actividad de Ejemplo", proporciona referencias que describen ensayos los cuales se pueden utilizar para probar la actividad terapéutica y/o biológica de una proteína Terapéutica: X o una proteína de fusión de albúmina que comprende una porción de proteína terapéutica X (o fragmento de la misma). Cada una de las referencias citadas en la columna "Ensayo de Actividad de Ejemplo" son citadas en la presente como referencia en su totalidad, particularmente con respecto a la descripción del ensayo de actividad respectivo descrito en la referencia (ver la sección de Métodos, por ejemplo) para probar la correspondiente actividad biológica divulgada en la columna "Actividad Biológica" de la Tabla 1. La cuarto columna, "Indicación Preferida: Y", describe enfermedades, trastornos, y/o condiciones que se pueden tratar, prevenir, dianosticar, y/o aliviar mediante la proteína Terapéutica X o una proteína de fusión de albúmina que comprende una porción de proteína Terapéutica X (o fragmento de la misma). La columna "Constructo ID" (columna 5) proporciona un enlace a un constructo de fusión de albúmina de ejemplo divulgado en la Tabla 2 el cual codifica una proteína de fusión de albúmina que comprende, o alternativamente consta de la porción de Proteína Terapéutica X (o fragmento de la misma).

Tabla 1

Proteína Terapéutica X	Actividad Biológica	Ensayo de Actividad de Ejemplo	Indicación Preferida Y	Constructo ID	Proteína Terapéutica Z
Hormona de crecimiento humano (Pegvisamont, Somatrem, Somatropina TROVERT, PROTROPIN BIO-TROPIN, HUMATROPE, NUTROPIN, NUTROPIN AQ, NUTROPHIN, NORDITROPIN, GENOTROPIN, SAIZEN, SEROSTIM)	Se une a dos moléculas GHR e induce transducción de señales a través de dimerización de receptores	Ensayo de proliferación de Ba/F3-hGHR, un bioensayo específico novedoso para hormona de crecimiento humano de suero. J Clin Endocrinol Metab 2000 Nov, 85(11) 4274-9 Inmunoensayo de hormona de crecimiento (GH) de plasma y bioensayo tibial Appl Physiol 2000 Dic, 89(6) 2174-8 (hGH) proliferación mediada por receptores de hormona de crecimiento (hGH) Growth Horm IGF Res 2000 Oct 10(5) 248-55 Estándar internacional para hormona de crecimiento, Horm Res 1999, 51 Suppl 1 7 12	Acromegalia, falla de crecimiento, reemplazo de hormona de crecimiento, deficiencia de hormona de crecimiento Deficiencia de Hormona de Crecimiento Pediátrica, Deficiencia de Hormona de Crecimiento Adulta, Deficiencia de Hormona de Crecimiento Yodopática retardo de crecimiento, Síndrome de Prader-Willi, síndrome de Prader-Willi en niños de 2 años o mayores, deficiencias de crecimiento, falla de crecimiento asociada con insuficiencia renal crónica, Osteoporosis, Osteoporosis Postmenopáusica, Osteopenia, Osteoclastogénesis quemaduras, Cachexia Cachexia de Cáncer Enanismo, Trastornos Metabólicos Obesidad, Falla Renal, Síndrome de Turner Fibromialgia, Tratamiento de Fracturas, flaqueza Agotamiento por SIDA, Agotamiento Muscular; Estatura Corta Agentes Diagnósticos Infertilidad en la mujer, lipodistrofia	3468, 3469 3470, 3475	Ver Tabla 2, SEQ ID NO Z para constructo particular
Interferona alfa ((Interferona alfa 2b,	Confiere un rango de respuestas celulares,	Ensayo antiviral Rubinsein S, Familletti PC,	Infecciones virales incluyendo síndrome respiratorio agudo	2249 2343 2366, 2381,	Ver, Tabla 2, SEQ ID N Z para constructo

recombinante, Interferona alfa n1, Interferona alfa n3, Peginterferona alfa 2b Ribavirina e Interferona alfa 2b Interferona alfacon 1, interferona consenso YM 643, CIFN, interferona –alfa consenso, inteferona de consenso de metionilo recombinante, interferona de consenso recombinante· CGP 35269 RO 253036, RO 258310, INTRON A, PEG INTRON, OIF, OMNIFERON, PEG-ONMIFERON, VELDONA PEG-REBETRON, ROFERON A WELLFERON, ALFERON N/LDO, REBETRON, ALTEMOL, VIRAFERONPEG, PEGASYS, VIRAFERON, VIRAFON, AMPLIGEN, INFERGEN, INFAREX ORAGEN)	incluyendo actividades antivirales, antiproliferativas, antitumorales, e inmunomoduladoras, estimulan la producción de dos enzimas una proteinquinasa y una oligoadenilato sintetasa.	Pestka S (1981) ensayo conveniente para interferonas. J Virol. 37(2) 755-8, ensayo de antiproliferación Gao Y (1999) Sensibilidad de una línea tumoral positiva a virus epstein barr Daule, a interferona alfa correlaciona con expresión de un transcripto viral rico en GC Mol Cell Biol. 19(11) 7305-13	severo (SARS) y otras infecciones por coronavirus, filovirus, incluyendo pero sin limitar a virus Ebola y virus Marburg Arenavirus, incluyendo pero sin limitar a virus Pichende, virus Lassa, virus Machupo, virus Guanarito y virus de coriomeningitis linfocítico (LCMV), Bunyavirus, incluyendo pero sin limitar a virus Punta toro, virus de fiebre hemorrágica Crimean Congo, virus de fiebre de mosca de arena, virus de fiebre del Valle de Rift, virus La crosse, y hantavirus, flavivirus, incluyendo pero sin limitar a fiebre amarilla, virus Banzi, virus del Nilo occidental, virus de dengue, virus de Encefalitis Japonesa, encefalitis transmitida por garrapata, fiebre hemorrágica Omsk, y virus de Enfermedad Forestal de Kyasanur· Togavirus, incluyendo pero sin limitar a virus de encefalitis equina venezolana, oriental y occidental, virus de Ross River; Orthopoxvirus, incluyendo pero sin limitar Vaccinia, viruela vacuna viruela, y viruela de los monos, virus herpes, FluA/B, virus sincitial respiratorio (RSV), sarampión, rinovirus, adenovirus, virus Semliki Forest, fiebres hemorrágicas	2382, 2410, 3165, 3422 3423, 3424, 3476.	particular

			virales, rabdovirus, paramixovirus, incluyendo pero sin limitar a Nipah virus y virus Hendra, y otros agentes virales identificados por los centros para control y prevención de enfermedades como agentes patológicos de alta prioridad (es decir, agentes Categoría A, B y C, ver *ej.* Moran, Emerg. Med. Clin. North Am., 2002, 20(2) 311-30 y Darling, Emerg. Med. Clin North Am. 2002, 20(2) 273-309).		
Interferona beta (interferona beta 1a, Interferona beta 1b, Interferona beta serina, SH 579 ZK 157046 BCDF, beta 2 IF, Interferona-beta 2 rhIL-6, SJ0031, DL 8234, FERON, IFNbeta, BETASERON AVONEX, REBIF BETAFERON, SIGOSIX)	Modula expresión de antígenos MHC, actividad de células NK y producción iFNg y producción de IL12 en monolitos.	Ensayo antiviral Rubinstein S, Familletti PC, Pestka S. (1981) Ensayo conveniente para interferonas. J Virol. 37(2) 755-8, Ensayo antiproliferación Gao Y, (1999)) Sensibilidad de una línea tumoral positiva a virus epstein barr, Daule, a interferona alfa correlaciona con expresión de un transcripto viral rico en GC Mol Cell Biol. 19(11) 7305-13	Infecciones virales incluyendo síndrome respiratorio agudo severo (SARS) y otras infecciones por coronavirus, filovirus, incluyendo pero sin limitar a virus Ebola y virus Marburg, Arenavirus, incluyendo pero sin limitar a virus Pichende, virus Lassa, virus Machupo, virus Guanarito, y virus de coriomeningitis linfocítico (LCMV), Bunyavirus, incluyendo pero sin limitar a virus Punta toro, virus de fiebre hemorrágica Crimean Congo, virus de fiebre de mosca de arena, virus de fiebre del Valle de Rift, virus La crosse, y hantavirus, flavivirus, incluyendo pero sin limitar a fiebre amarilla, virus Banzi, virus del Nilo occidental, virus de dengue, virus de Encefalitis Japonesa,	1778, 1779, 2011, 2013, 2053, 2054, 2492, 2580, 2795, 2796, 2797	Ver Tabla 2, SEQ ID NO Z para constructo particular

			encefalitis transmitida por garrapata, fiebre hemorrágica Omsk, y virus de Enfermedad Forestal de Kyasanur; Togavirus, incluyendo pero sin limitar a virus de encefalitis equina venezolana, oriental y occidental, virus de Ross River, Orthopoxvirus, incluyendo pero sin limitar Vaccinia, viruela vacuna, viruela, y viruela de los monos, virus herpes, FluA/B, virus sincitial respiratorio (RSV), sarampión, rinovirus, adenovirus, virus Semliki Forest, fiebres hemorrágicas virales, rabdovirus, paramixovirus, incluyendo pero sin limitar a Nipah virus y virus Hendra, y otros agentes virales identificados por los centros para control y prevención de enfermedades como agentes patológicos de alta prioridad (es decir, agentes Categoría A, B y C, ver, *ej.* Moran, Emerg, Med. Clin. North Am., 2002, 20(2) 311-30 y Darling, Emerg. Med Clin North Am 2002, 20(2) 273-309).		
Péptido natrirético (BNP, péptido natriurético de cerebro)	Estimula relajación y vasodilatación de músculos lisos, natriuresis y supresión de endotelina	La inhibición de angiotensina se puede determinar utilizando ensayos conocidos en el estado de la técnica, por ejemplo utilizando un ensayo de proliferación in Vitro con fibroblastos	Falla cardiaca congestiva, sobrecarga de volumen cardiaco, descompensación cardiaca, Falla Cardiaca, Disfunción Ventricular Izquierda, Disnea Hipertrofia glomerular, lesión glomerular, enfermedad	3618, 3690, 3691, 3715, 3723, 3724, 3725, 3736, 3769, 3778, 3783, 3795, 3796, 3809, 3896, 3897,	Ver Tabla 2, SEQ ID NO Z para constructo particular

		cardiacos de rata como se describió en Naunyn Schmiedebergs Arch Pharmacol 1999 May, 359(5) 394-9 La vasodilación se puede medir en animales al medir las respuestas biogénicas de arterias renales pequeñas en un sistema arteriográfico isobárico (*ver* Am J Physiol Regul Integr Comp Physiol 2002 Aug, 283(2) R349-R355). Natriuesis es determinada al medir la cantidad de sodio en la orina.	glomerular renal, Falla renal aguda.	3898, 3899, 3900.	
Isoforma c de BDNF (factor neurotrófico derivado de cerebro)	Factor neurotrófico que promueve crecimiento neuronal, diferenciación y supervivencia, mantiene la supervivencia de subgrupos de neuronas periféricas y centrales durante desarrollo, juega un papel en una función del sistema nervioso adulto, puede contribuir a las respuestas nociceptivas.	La actividad BDNF sobre crecimiento neuronal se puede medir utilizando ensayos de crecimiento neuronal y actividad sinaptica, tales como aquellos descritos en Bartrup (1997) Neuroreport 1 8(17).3791 4, actividad BDNF sobre recepción se puede evaluar al medir conductas nociceptivas, hiperalgesia, y/o alodinia como se describió en Shu Dolor (1999) 80 463-470 y en Zhou Eur J Neurosci (2000) 12 100-105.	Dolor Dolor neuropático, Síndrome I de dolor regional complejo, Distrofia simpatética de reflejo neuralgia trigeminal Alodinia, hiperalgesia primaria y/o secundaria, Causalgia, dolor de extremidad fantasma, dolor post-quirúrgico, Síndrome de pies ardientes síndrome de Guillain-Barre, síndrome talámico, dolor post-apoplejía, dolor vasculítico/angiopático, Dolor yodopático, Dolor asociado con cualquiera de lo siguiente neuropatía de entrampado, transección nerviosa, Lesión de médula espinal, Formación de cicatrices, neuropatía alcohólica, pelagra beriberi, neuralgia post herpética, dolor	3549	Ver Tabla 2, SEQ ID NO Z para constructo particular

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			de VIH/SIDA, Neurotoxicidad de Vincristina, Neurotoxicidad de cisplatina, Neurotoxicidad de taxol, Neurotoxicidad de talio, Neurotoxicidad de arsénico, Terapia de radiación, Diabetes, Malignidades, Esclerosis Múltiple, enfermedad de Fabry enfermedad de Tangier, o Amiloide.		
Péptido 1 Tipo Glucagon (GLP1, GLP-1, Insulinotropina)	Estimula la síntesis y liberación de insulina mejora la sensibilidad de tejidos adiposos, musculares y hepáticos hacia insulina, estimula absorción de glucosa, retarda el proceso digestivo suprime el apetito, bloquea la secreción de glucagon	La actividad GLP1 se puede evaluar in Vitro utilizando un ensayo de absorción de [3-H]-glucosa (J Biol Chem 1999 Oct 22, 274(43) 30864-30873). Los efectos de GLP-1 sobre el aprendizaje se pueden investigar utilizando la abstinencia positiva y paradigmas de laberinto de agua Morris en ratas (Brain Res. 1996, 716 29-38 y Nature 1982, 2937 681 683).	Hiperglicemia, Diabetes, Diabetes Insipidus, Diabetes mellitus, Diabetes tipo 1, Diabetes tipo 2, Resistencia a insulina Deficiencia de insulina, Hiperlipidemia, Hipercetonemia, Diabetes mellitus no dependiente de insulina (NIDDM), Diabetes mellitus dependiente de insulina (IDDM), Una condición asociada con diabetes incluyendo, pero sin limitar a Obesidad, Enfermedad Cardiaca, Hiperglicemia, Infecciones, Retinopatía y/o Ulceras, Trastornos Metabólicos, Trastornos Inmunes, obesidad, Trastornos vasculares, Supresión de Peso Corporal, Supresión de Apetito, Síndrome X, Deterioro Cognitivo, Pérdida de Memoria. Utilizar para mejorar aprendizaje, memoria, aprendizaje asociativo, aprendizaje espacial, plasticidad	3610, 3696	Ver Tabla 2, SEQ ID NO Z para constructo particular

			hipocampal, conciencia, y/o neuroprotección		
Glucocerebrosidasa lisosomal, (Algucerasa, β-glucocerebrosidasa, β-D-glucosil-*N* acilesfingosina glucohirdolasa, Glucosilceramidasa, β-glucosidasa ácida, Imiglucerasa, CEREDASE, CEREZYME) (GCB)	Cataliza la hidrólisis de D-glucosil-*N* acilesfingosina a D-glucosa y N-acilesfingosina , cataliza la hidrólisis de glucocerebrocidad a ceramida de glucosa	La actividad enzimatica se puede evaluar utilizando métodos conocidos en el estado de la técnica, por ejemplo, el ensayo de cromatografía de capa delgada descrita por Poulos, Clin Chim Acta. 1976 Nov 72(3) 327-335, o el método FACS descrito por Rudensky, Blood Cells Mol. Dis. 2003 Enero-Febrero, 30(1) 97-99	Enfermedad de Gaucher, enfermedades de almacenamiento liposomal	3920-3921, 3922, 3923,	Ver Tabla 2, SEQ ID NO Z para constructo particular También, para secuencias de glicocerebrosidasa liposomal particular o variante de las mismas, ver GLM_HUMAN, Número de Acceso Genbank. P04062, CAS-143003-46-7, cada una de estas incorporada en la presente como referencia Otras secuencias de glucocerebrosidasa son divulgadas en la Publicación de Patente No. 2004009165, 20030133924, 20020127219, cada una de estas es incorporada en la presente como referencia Glucocerebrosidasas lisosomales adicionales son divulgadas en las Patentes Americana No. 6.696.272, 5.879.680, 5.549,892, cada una de estas incorporada en la presente como referencia.
Arginina deiminasa (ADI-SS)	Cataliza la hidrólisis irreversible de arginina a citrulina y	La actividad enzimática se puede medir utilizando métodos bien conocidos en	Cáncer, incluyendo pero sin limitar a melanoma y carcinoma hepatocelular	3910, 3915, 3917, 3918.	Ver Tabla 2, SEQ ID NO Z para constructo particular También

	amoniaco, inhibe síntesis de óxido nítrico inhibe angiogenesis, inhibe proliferación de numerosas células tumorales.	el estado de la técnica tal como el ensayo enzimático descrito en Weickmann, J Biol. Chem. 1977 Apr 252(8) 2615-2620. La inhibición de angiogénesis se puede evaluar utilizando métodos conocidos en el estado de la técnica, por ejemplo, el ensayo de Matrigel como se describió en Park. Br J Cáncer 2003 Sept, 89(5) 907-914. La inhibición de producción de oxido nítrico se puede evaluar utilizando métodos conocidos en el estado de la técnica por ejemplo, como se describe en Noh, Mol. Cells 2002 Feb 13(1) 137 143 La proliferación de células tumorales se puede evaluar utilizando métodos conocidos en el estado de la técnica, por ejemplo, el ensayo de proliferación como se describió en Miyazaki, Cáncer Res. 1990 Aug, 50(15) 4522 4527	Cánceres adicionales incluyen, pero no están limitados a carcinoma de células escamosas, osteosarcoma, glioma/astrocitoma, glioblastoma, leucemia promielocítica, leucemia linfoblástica, y carcinomas de la cerviz, mama, ovarios, próstata, colon o pulmón		para secuencia particular de arginina deiminasa o variante de la misma, ver Baur, Eur, J Biochem 1989 Enero, 179(1) 53-60, Misawa J Biotechnol. 1994, 361 145-155, cada una de estas incorporada en la presente como referencia. Otras secuencias de arginina deiminasa son divulgadas en la Patente Americana No. 20040096437 incorporada como referencia. Otras arginina deiminasas son divulgadas en Patentes Americanas No 6.180.387, 5.804.183, 5.474.928, 5.196.195, cada una de estas incorporada en la presente como referencia
Uricasa (oxidasa de urato, *Aspergillis flavus* uricasa, *Candida utilis* uricasa, rasburicasa FASTURTEC, ELITEK)	Cataliza la oxidación de ácido úrico en alantoina	Los niveles de ácido urico se pueden medir espectrofotometricamente, colorimetricamente o utilizando otros métodos que son bien conocidos en el estado de la técnica, tal	Gota, hiperuricemia, hiperurecia de lisis tumoral, hiperuricemia inducida por quimioterapia, apoplejía isquémica, anormalidades lípidas, trastornos cardiovasculares, incluyendo	3903, 3904, 3936, 3937, 3938, 3939, 3940, 3941, 3942, 3943, 3944.	Ver Tabla 2, SEA ID NO Z para constructo particular También, para secuencia de uricasa particular o variante de la misma, ver CAS-134774-45.1,

		como en Kageyama Clin Chim. Acta. 1971 31(2) 421-426.	pero sin limitar a hipertensión, enfermedad arterial coronaria, aterosclerosis.		Wu P N A.S. USA 1989 Dic, 86(23) 9412 9416, cada una de las anteriores incorporada en la presente como referencia Otras secuencias de uricasa son divulgadas en las Patentes Americanas No. 4 062 731, 4.273.874, 4.987 076, 5.376.545, 5.700.674, 5.728.562, 5.801 036, 5.834.273, 5.955.336, cada una de estas es incorporada en la presente como referencia
Híbridos de interferona, específicamente preferidos híbrido IFNalfa A/D (versión BglII) Híbrido IFNalfa A/D (versión PvuII) Híbrido IFNalfa A/F Híbrido IFNalfa A/B Híbrido IFNbeta 1/alfa D (híbrido IFNbeta-1/alfa 1) Híbrido IFNalfa/beta	Confieren un rango de respuestas celulares incluyendo actividades antivirales, antiproliferativas, antitumorales e inmunomoduladoras, estimulan producción de dos enzimas una proteinquinasa y una oligoadenilato sintasa También, modula expresión de antígenos MHC, actividad de células NK y producción IFNg y producción IL12 en monolitos.	Ensayo anti-viral Rubinstein S, Familletti PC, Pestka S. (1981) Ensayo conveniente para interferonas. J Virol. 37(2) 755-8, ensayo de antiproliferación Gao Y (1999). Sensibilidad de una línea tumoral positiva a virus epstein-barr, Daule, a interferona alfa correlaciona con expresión de un transcripto viral rico en GC Mol Cell Biol. 19(11) 7305-13	Infecciones virales incluyendo síndrome respiratorio agudo severo (SARS) y otras infecciones por coronavirus, filovirus, incluyendo pero sin limitar a virus Ebola y virus Marburg; Arenavirus, incluyendo pero sin limitar a virus Pichende, virus Lassa, virus Machupo, virus Guanarito, y virus de coriomeningitis linfocítico (LCMV) Bunyavirus, incluyendo pero sin limitar a virus Punta toro, virus de fiebre hemorrágica Crimean Congo, virus de fiebre de mosca de arena, virus de fiebre del Valle de Rift, virus La crosse, y hantavirus, flavivirus, incluyendo pero sin limitar a fiebre amarilla, virus	2872, 2873 2874, 2875, 2876.	Ver Tabla 2, SEQ ID NO Z para constructo particular

			Banzi, virus del Nilo occidental, virus de dengue, virus de Encefalitis Japonesa, encefalitis transmitida por garrapata, fiebre hemorrágica Omsk, y virus de Enfermedad Forestal de Kyasanur, Togavirus, incluyendo pero sin limitar a virus de encefalitis equina venezolana, oriental y occidental, virus de Ross River, Orthopoxvirus, incluyendo pero sin limitar Vaccinia, viruela vacuna, viruela, y viruela de los monos, virus herpes, FluA/B, virus sincitial respiratorio (RSV), sarampión, rinovirus, adenovirus, virus Semliki Forest; fiebres hemorrágicas virales, rabdovirus paramixovirus, incluyendo pero sin limitar a Nipah virus y virus Hendra, y otros agentes virales identificados por los centros para control y prevención de enfermedades como agentes patológicos de alta prioridad (es decir, agentes Categoría A, B y C, ver, *ej.* Moran, Emerg, Med. Clin North. Am., 2002, 20(2) 311-30 y Darling, Emerg. Med Clin North Am. 2002, 20(2) 273-309)		
IL-2 (Aldesleucina, interleucina 2 tóxina de fusión, factor de crecimiento de células T, PR LEUKIN,	Promueve el crecimiento de células B y T y aumenta la actividad de destrucción de células	Ensayo de proliferación de células T "Actividad biológica de interleucina-2 humana recombinante producida por Escherichia	Cáncer; tumores sólidos, Cáncer pancreatico, Cáncer de colon, Cáncer hepático, Linfomas-T, Enfermedad injerto vs. Huésped (GVH),	1757, 1758, 1812, 1813, 1952 1954.	Ver Tabla 2, SEQ ID NO Z para constructo particular

IMMUNAGE, CELEUK, ONCOLIPIN 2, MACROLIN)	NK y CTL.	coli." Science 223 1412-1415, 1984. Ensayo de citotoxicidad CTL y de células destructoras naturales (NK) "Control de homeostasis de células T de memoria CD8+ mediante citoquinas opuestas. Science 288 675-678, 2000, CTLL-2 Proliferación Gillis (1978) J Immunol. 120, 2027	enfermedades autoinmunes, trastornos autoinmunes, malignidades positivas de receptores de IL-2		
Adrenomedulina (ADM)	Inhibe Na+-K+-ATPasa renal, aumenta síntesis de prostaglandina E2 que regula contracción y relajación de músculo liso, así como también la dilatación y constricción de vasos sanguíneos inhibe secreción de aldosterona produce diuresis y natriuresis. Se relaciona con regulación de presión sanguínea y balance de sal/agua/electrolitos en fluidos corporales, renoprotección	La actividad de Na+-K+-ATPase renal se puede medir utilizando ensayos conocidos en el estado de la técnica, tal como en Ku, 1987, Endocrinología 120.2166-2173 La vasodilatación se puede medir utilizando ensayos conocidos en el estado de la técnica (Ashton Pharmacology 2000, 61(2) 101 105. La síntesis de prostaglandina E2 se puede determinar utilizando ensayos conocidos en el estado de la técnica, (Cheng, J Endocrinol 2004 Agost, 182(2) 249-56). Los niveles de aldosterona se pueden medir utilizando métodos conocidos en el estado de la técnica, por ejemplo, en Yamato, Circ J 2003 May, 67(5) 384-90. La presión sanguínea se puede medir con un esfigmomanómetro	Tratamiento de falla cardiaca congestiva, Hipertensión, enfermedad cardiovascular, falla renal aguda, necrosis tubular aguda, enfermedad renal, enfermedad glomerular renal, carga de volumen cardiaco, descompensación cardiaca disfunción ventricular izquierda, disnea. Tratamiento para niveles elevados de aldosterona, los cuales pueden llevar a vasoconstricción, salida cardiaca deteriorada y/o hipertensión, falla cardiaca, lesión de repercusión micardial, remodelación de ventrículo izquierdo.	3924, 3925, 3926, 3927	Ver Tabla 2, SEQ ID NO Z para constructo particular Ver también, Vesely Am J Physiol Renal Physiol 2003, 285 F167-177 el cual es incorporado en la presente como referencia

		o utilizando otros métodos conocidos en el estado de la técnica, tal como en Reddy Ultrasound Med N,Biol 2003 Marzo, 29(3) 379-85. Natriuresis es determinada al medir la cantidad de sodio en la orina Diuresis se determina al medir la cantidad de orina secretada.			
Péptido natriurético de acción prolongada (LANP proANP-(31 67)	Inhibe Na+-K+-ATPasa renal, aumenta síntesis de prostaglandina E2 que regula contracción y relajación de músculo liso, así como también la dilatación y constricción de vasos sanguíneos, inhibe secreción de aldosterona produce diuresis y natriuresis. Se relaciona con regulación de presión sanguínea y balance de sal/agua/electrolitos en fluidos corporales, renoprotección	La actividad de Na+-K+-ATPase renal se puede medir utilizando ensayos conocidos en el estado de la técnica, tal como en Ku, 1987, Endocrinología 120 2166-2173 La vasodilatación se puede medir utilizando ensayos conocidos en el estado de la técnica (Ashton Pharmacology 2000 61(2) 101 105. La síntesis de prostaglandina E2 se puede determinar utilizando ensayos conocidos en el estado de la técnica, (Cheng, J Endocrinol 2004 Agost; 182(2) 249-56). Los niveles de aldosterona se pueden medir utilizando métodos conocidos en el estado de la técnica, por ejemplo, en Yamato, Circ J 2003 May, 67(5) 384-90. La presión sanguínea se puede medir con un esfigmomanómetro o utilizando otros métodos	Tratamiento de falla cardiaca congestiva, Hipertensión enfermedad cardiovascular; falla renal aguda, necrosis tubular aguda, enfermedad renal, enfermedad glomerular renal, carga de volumen cardiaco descompensación cardiaca disfunción ventricular izquierda, disnea Tratamiento para niveles elevados de aldosterona, los cuales pueden llevar a vasoconstricción, salida cardiaca deteriorada y/o hipertensión, falla cardiaca, lesión de repercusión micardial, remodelación de ventrículo izquierdo.	3888, 3889	Ver Tabla 2, SEQ ID NO Z para constructo particular Ver también, Vesely Am J Physiol Renal Rhysiol 2003 285 F167-177 el cual es incorporado en la presente como referencia

		conocidos en el estado de la técnica, tal como en Reddy Ultrasound Med N,Biol 2003 Marzo, 29(3) 379-85. Natriuresis es determinada al medir la cantidad de sodio en la orina. Diuresis se determina al medir la cantidad de orina secretada.			
Dilatador de Vasos (VDP, proANP-(79-98)).	Inhibe Na+-K+-ATPasa renal, aumenta síntesis de prostaglandina E2 que regula contracción y relajación de músculo liso, así como también la dilatación y constricción de vasos sanguíneos, inhibe secreción de aldosterona, produce diuresis y natriuresis. Se relaciona con regulación de presión sanguínea y balance de sal/agua/electrolitos en fluidos corporales, renoprotección.	La actividad de Na+-K+-ATPase renal se puede medir utilizando ensayos conocidos en el estado de la técnica, tal como en Ku, 1987, Endocrinología 120 2166-2173 La vasodilatación se puede medir utilizando ensayos conocidos en el estado de la técnica (Ashton Pharmacology 2000, 61(2) 101 105. La síntesis de prostaglandina E2 se puede determinar utilizando ensayos conocidos en el estado de la técnica, (Cheng, J Endocrinol 2004 Agost; 182(2) 249-56). Los niveles de aldosterona se pueden medir utilizando métodos conocidos en el estado de la técnica por ejemplo, en Yamato, Circ J 2003 May, 67(5) 384-90 La presión sanguínea se puede medir con un esfigmomanómetro o utilizando otros métodos conocidos en el estado de la	Tratamiento de falla cardiaca congestiva, Hipertensión, enfermedad cardiovascular; falla renal aguda, necrosis tubular aguda, enfermedad renal, enfermedad glomerular renal carga de volumen cardiaco, descompensación cardiaca disfunción ventricular izquierda, disnea. Tratamiento para niveles elevados de aldosterona, los cuales pueden llevar a vasoconstricción, salida cardiaca deteriorada y/o hipertensión, falla cardiaca, lesión de repercusión micardial, remodelación de ventrículo izquierdo	3888, 3889	Ver Tabla 2, SEQ ID NO Z para constructo particular Ver también, Vesely Am J Physiol Renal Rhysiol 2003, 285 F167 177 el cual es incorporado en la presente como referencia

		técnica, tal como en Reddy Ultrasound Med N¡Biol 2003 Marzo, 29(3) 379-85. Natriuresis es determinada al medir la cantidad de sodio en la orina Diuresis se determina al medir la cantidad de orina secretada.			
Péptido Calliurético (KUP proANP-(99-126)).	Involucrado en regulación de presión sanguínea y balance de sal/agua/electrolitos en fluidos corporales.	La presión sanguínea se puede medir con un esfigmomanómetro o utilizando otros métodos conocidos en el estado de la técnica, tal como en Reddy Ultrasound Med N¡Biol 2003 Marzo, 29(3) 379-85. Natriuresis es determinada al medir la cantidad de sodio en la orina. Diuresis se determina al medir la cantidad de orina secretada	Tratamiento de falla cardiaca congestiva, Hipertensión, enfermedad cardiovascular; falla renal aguda, necrosis tubular aguda enfermedad renal, enfermedad glomerular renal.	3890, 3891	Ver Tabla 2, SEQ ID NO Z para constructo particular Ver también, Vesely Am J Physiol Renal Rhysiol 2003, 285 F167 177 el cual es incorporado en la presente como referencia
Péptido Natriurético Tipo C (CNP)	Promueve diuresis y natriuresis. Involucrado en regulación de presión sanguínea y balance de sal/agua/electrolitos en fluidos corporales.	Natriuresis es determinada al medir la cantidad de sodio en la orina. Diuresis se determina al medir la cantidad de orina secretada.	Tratamiento de falla cardiaca congestiva Hipertensión enfermedad cardiovascular; falla renal aguda, necrosis tubular aguda, enfermedad renal, enfermedad glomerular renal.	3892, 3893	Ver Tabla 2, SEQ ID NO Z para constructo particular Ver también, Vesely Am J Physiol Renal Rhysiol 2003, 285 F167 177 el cual es incorporado en la presente como referencia.
Péptido natriurético *dendroaspis* (DNP)	Inhibe Na+-K+-ATPasa renal, aumenta síntesis de prostaglandina E2 que regula contracción y relajación de músculo liso, así como también la dilatación y	La actividad de Na+-K+-ATPase renal se puede medir utilizando ensayos conocidos en el estado de la técnica, tal como en Ku, 1987, Endocrinología 120 2166-2173. La vasodilatación se puede	Tratamiento de falla cardiaca congestiva, Hipertensión, enfermedad cardiovascular, falla renal aguda, necrosis tubular aguda, enfermedad renal enfermedad glomerular renal, carga de volumen cardiaco, descompensación	3894, 3895.	Ver Tabla 2, SEQ ID NO Z para constructo particular Ver también, Vesely Am J Physiol Renal Rhysiol 2003 285 F167-177 el cual es incorporado en la presente como

	constricción de vasos sanguíneos, inhibe secreción de aldosterona, produce diuresis y natriuresis. Se relaciona con regulación de presión sanguínea y balance de sal/agua/electrolitos en fluidos corporales, renoprotección	medir utilizando ensayos conocidos en el estado de la técnica (Ashton Pharmacology 2000, 61(2) 101-105. La síntesis de prostaglandina E2 se puede determinar utilizando ensayos conocidos en el estado de la técnica, (Cheng, J Endocrinol 2004 Agost; 182(2) 249-56). Los niveles de aldosterona se pueden medir utilizando métodos conocidos en el estado de la técnica, por ejemplo, en Yamato, Circ J 2003 May, 67(5) 384-90. La presión sanguínea se puede medir con un esfigmomanómetro o utilizando otros métodos conocidos en el estado de la técnica, tal como en Reddy Ultrasound Med N¡Biol 2003 Marzo, 29(3) 379-85. Natriuresis es determinada al medir la cantidad de sodio en la orina Diuresis se determina al medir la cantidad de orina secretada.	cardiaca disfunción ventricular izquierda, disnea. Tratamiento para niveles elevados de aldosterona los cuales pueden llevar a vasoconstricción, salida cardiaca deteriorada y/o hipertensión, falla cardiaca, lesión de repercusión micardial, remodelación de ventrículo izquierdo.		referencia
TweakR (Fn 14, factor de crecimiento de fibroblastos-inducible 14)	Estimula muerte celular apoptótica, induce angiogénesis, promueve migración y proliferación de células endoteliales, cuando muta produce susceptibilidad a síndrome Klippel-	La muerte celular apoptotica se puede medir utilizando ensayos conocidos en el estado de la técnica, por ejemplo, en Nakayama, J Immunol. 2003 Enero 1, 170(1) 341 8). La migración de células endoteliales y proliferación	Tratamiento de síndrome de Klippel-Trenaunay; Cáncer; Tumores sólidos, Cáncer Pancreatico Cáncer de colon, Cáncer hepático, Cáncer Cerebral, Cáncer gástrico Cáncer pulmonar, Cáncer de mama, Cáncer óseo, enfermedades autoinmunes,	3919, 3932	SEQ ID NO Z para constructo particular

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	Tremaunay	se puede determinar utilizando métodos conocidos en el estado de la técnica, por ejemplo, en Woods J Biol Chem. 2002 Enero 18, 277(3) 1924-7	trastornos autoinmunes, Lupus, artritis reumatoide, esclerosis múltiple, trastornos de angiogénesis, enfermedad cardiovascular		
Kiss-1 (Metastatina, kisspeptina 54)	Suprime metástasis tumoral, activa receptor acoplado a proteína G GPR54 para inhibir progresión tumoral, controla secreción de gonadotropina.	La activación de GPR54 se puede determinar utilizando ensayos conocidos en el estado de la técnica, por ejemplo, en Becker, Biochem Biophys Res Commun 2005 Enero 21, 326(3) 677-86. Secreción de gonadotropina se puede determinar al medir la cantidad de hormonas de gonadotropina en el suero.	Tratamiento de Cáncer, Carcinoma Gástrico, Carcinomas de Mama, Carcinomas de Células Escamosas Esofágicas Cáncer de Vejiga, Tumores Sólidos, Cáncer de Tiroides, Cáncer Pancreatico, Cáncer de Colon, Cáncer Hepático, Cáncer pulmonar; Cáncer Cerebral, Cáncer Óseo, Deficiencia de gonadotropina, infertilidad, Hipopituitarismo Panhipopituitarismo, Oligomenorrea, Amenorrea pérdida de libido, ráfagas calientes, Dispaneuria, impotencia, Osteopenia, hipogonadismo hipogonadotrópico, síndrome de Kallmann,g hipogonadismo secundario.g	3928, 3929	Ver Tabla 2, SEQ ID NO Z para constructo particular También ver, Dhar., Int J Cáncer 2004 Oct 10, 111(6) 868-72, Harás, Clin Exp Metastasis. 2003, 20(1) 11-8, o Gottsch, Endocrinología. 2004 Sept 145(9) 4073-7 las cuales se incorporan como referencia
Efrin B1	Regula crecimiento tumoral y angiogenesis, regula migración e invasión de células cancerígenas, aumenta agregación de plaquetas y maduración de coagulos, Estimula proliferación de células T, producción	Crecimiento tumoral, angiogénesis, migración e invasión se pueden medir utilizando ensayos conocidos en el estado de la técnica (Nakada, Cáncer Res. 2004 Mayo 1, 64(9) 3179-85 o Noren, Proc Natls Acad Sci USA 2004 Abril 13, 101(15) 5583-8). La producción de células	Cáncer; Tumores sólidos, Cáncer Pancreático, Cáncer de colon, Cáncer hepático, Cáncer cerebral, Cáncer colorectal, Cáncer gástrico, Cáncer pulmonar, Cáncer Óseo, Cáncer de mama, Enfermedad cardiovascular; carcinoma hepatocelular Linfomas-T, enfermedad de Injerto vs. Huésped (GVH), enfermedades autoinmunes,	3934, 3935.	SEQ ID NO Z para constructo particular

	de linfocinas y actividad CTL, Estimula migración y proliferación de células endoteliales, requerida para función coclear normal	T, producción de linfoquinas, y actividad CTL se puede determinar utilizando ensayos conocidos en el estado de la técnica, por ejemplo, en Yu J Biol. Chem. 2004, 279(53) 55531-55539 La migración y proliferación de células endoteliales se puede determinar utilizando métodos conocidos en el estado de la técnica, por ejemplo, en Woods, J Biol Chem. 2002 Enero 18, 277(3) 1924-7 La función coclear se puede determinar utilizando ensayos conocidos en el estado de la técnica (Howard, Hear Res. 2003 Abril, 178 (1-2) 118-30).	trastornos autoinmunes, Hemofilia, enfermedad de von Willebrand, trastornos de sangrado, trastornos de sangrado que resultan de trastornos de tejido conectivo, Leucemia, Mieloma, derivación de corazón/pulmon, diálisis de riñón, o inducida por fármacos, trastornos de angiogenesis, trastornos cocleares, pérdida auditiva, Vertigo, Tinitus, Llenura Aural, trastornos auditivos internos agudos y/o crónicos.		
B7 H3	Aumenta inmunidad tumoral, Regula activación y respuestas inmunes de células T	La inmunidad tumoral se puede medir mediante métodos conocidos en el estado de la técnica, por ejemplo, en Luo, J Immunol 2004 Nov 1, 173(9) 5445-50 La activación de células T se puede medir utilizando ensayos conocidos en el estado de la técnica (Chapoval, Nat Immunol. 2001 Marz, 2(3) 269-74).	Cáncer, Tumores Sólidos, Cáncer Pancreático, Cáncer de Colon, Cáncer Hepático, Cáncer de pulmón, Cáncer Óseo, enfermedad autoinmune, trastornos autoinmunes, enfermedades óseas osteolíticas, hipercalcemia de malignidad, dolor óseo inducido por malignidad.	3933.	SEQ ID NO Z para constructo particular

Tabla 2

No. de fusión	Construct o ID	Nombre de Constructo	Descripción	Vector de Expresió	SEQ ID	SEQ ID NO X	SEQ ID NO Z	SEQ ID NO A	SEQ ID NO B	Secuencia Guía

				n	NO Y					
1	3422	Pmac35 APDP HSA IFNa	Péptido señal de fosfatasa ácida seguido por IFNa y HSA madura.	pSAC35	149	117	181	213	214	Fosfatasa acida
2	3423	Pmac35 Invs..HSA.I FNa	Péptido señal de invertasa seguido por IFNa y HSA madura	pSAC35	150	118	182	215	216	Invertasa
3	3424	pSAC35 K Tsp.HSA I FNa	Péptido señal de toxina asesina seguido por IFNa y HSA madura	pSAC35	151	119	183	217	218	Toxina asesina
4	2249	pSAC5 IFNa2-HSA también de nominado pSAC23 IF Na2-HSA	IFNa2 madura fusionada corriente arriba de HSA madura y corriente debajo de la secuencia líder HSA/kex2	pSAC35	152	120	184	219	220	HSA/kex2
5	2343	pSAC35.IN V IFNA2 HS A	Interferona alfa2 madura fusionada corriente arriba de HSA y corriente abajo de péptido señal de invertasa	pSAC35	153	121	185	221	222	Invertasa
6	2366	pSAC35.M AF IFNa2 HSA	IFNa2 madura fusionada corriente arriba de HSA madura y corriente abajo de secuencia líder de factor de apareamiento alfa de levadura.	pSAC35	154	122	186	223	224	MFα-1
7	2381	pC4 HSA IFNa2(C17 E181)	Aminoácidos C17 a E181 de IFNa2 (fragmento mostrado como aminoácidos C1 a E165 de SEQ ID NO 618) fusionada corriente abajo de HSA	pC4	155	123	187	225	226	HSA
8	2382	pC4 IFNa2 -HSA	IFNa2 fusionada corriente arriba de HSA madura.	pC4	156	124	188	227	228	Secuencia guía IFNα2 nativa
9	2410	pSAC35IN V IFNa-HSA	IFNa2 madura fusionada corriente debajo de péptido señal de invertasa	pSAC35	157	125	189	229	230	Invertasa

			y corriente arriba de HSA madura.							
10	3165	pSAC35 también denominado CID 3165, pSAC35 HSA.IFNbeta	HSA fusionada corriente arriba de IFNalfa y corriente debajo de la secuencia líder HSA/kex2.	pSAC35	158	126	190			HSA/kex2
11	1778	pSAC35 IFNbeta.M22-N187 HSA	Residuos M22-N187 de IFNb de longitud completa (mostrada como M1 a N166 de SEQ ID NO 463) fusionada corriente arriba de HSA y corriente debajo de secuencia líder HSA/kex2.	pSAC35	159	127	191	231	232	HSA/kex2
12	1779	pSAC35 HSA IFNbeta M22-N187	Residuos M22-N187 de IFNb de longitud total (mostrada como M1 a N166 de SEQ ID NO 464) fusonada corriente debajo de HSA con secuencia líder HSA/kex2	pSAC35	160	128	192			HSA/kex2
13	2011	pC4 IFNb-HSA	IFNb de longitud completa fusionada corriente arriba de HSa madura.	pC4	161	129	193	233	234	Secuencia guía IFNb nativa
14	2013	pC4 HSA IFNb.M22-N187	Aminoácidos M22 a N187 de IFNb (fragmento mostrado como aminoácidos M1 a N166 de SEQ ID NO 527) fusionada corriente abajo de HSA.	pC4	162	130	194			HSA
15	2053	pEE12 IFNb-HSA también denominado Pee12 1 IFNbeta HSA	IFNb de longitud completa fusionada corriente arriba de HSA madura.	pEE12 1	163	131	195			Secuencia guía IFNb nativa

16	2054	pEE12 HS A IFNb	IFNb madura fusionada corriente debajo de HSA.	pEE12 1	164	132	196			HSA
17	2492	PC4.IFNb(deltaM22). HSA	INFbeta de longitud completa mutante fusionada corriente arriba de HSA madura El primer residuo de IFNbeta madura nativa (M22) ha sido suprimido.	pC4	165	133	197			Líder IFNβ nativa
18	2580	pC4.IFNb(deltaM22,C 38S) HSA	IFNb fusionada corriente arriba de HSA madura La IFNb utilizada en esta fusión carece del primer residuo de la forma madura de IFNb, el cual corresponde a M22 de SEQ ID NO 1687 También el aminoácido 38 de SEQ ID NO 1687 ha mutado de Cys a Ser	pC4	166	134	198			IFNβ nativa
19	2795	pC4 HSA(A14)-IFNb.M22-N187	La forma madura de IFNb es fusionada al extremo C de HSA, la cual contiene un péptido señal modificado, diseñado para mejorar procesamiento y homogeneidad	pC4	167	135	199			HSA (A14) modificada
20	2796	PC4 HSA(S 14)-IFNb.M22-N187	La forma madura de IFNb es fusionada al extremo-C de HSA, el cual contiene un péptido señal modificado, diseñado para mejorar procesamiento y homogeneidad.	pC4	168	136	200			HSA (4 14) modificada
21	2797	pC4 HSA (G14)-IFNb.M22-N187	La forma madura de IFNb es fusionada al extremo-C de HSA, el cual contiene el extremo-C de HSA, el cual contiene un péptido señal modificado.	PC4	169	137	201			HSA (G14) modificada

22	2872	pSAC35 HSA.IFNaA(C1 Q91)/D(L93 E166)	Este constructo contiene una forma híbrida de IFNaA y IFNaD fusionada corriente abajo de HSA madura.	pSAC35	170	138	202	235	236	HSA/kex2
23	2874	pSAC35 HSA.IFNaA(C1-Q91)/F(L93-E166)	Este constructo contiene una forma híbrida de IFNaA y IFNaB fusionada corriente abajo de HSA madura.	pSAC35	171	139	203	237	238	HSA/kex2
24	2874	pSAC35 HSA.IFNaA(C1 Q91)/F(L93-E166)	Este constructo contiene una forma híbrida de IFNaA y IFNaF fusionada corriente abajo de HSA madura	pSAC35	172	140	204	239	240	HSA/kex2
25	2875	pSAC35 HSA.IFNaA(C1Q-62)/D/Q64-E166)	Este constructo contiene una forma híbrida de IFNaA y IFNaD fusionada corriente abajo de HSA madura	pSAC35	173	141	205	241	242	HSA/kex2
26	2876	pSAC35 HSA.IFNaA(C1 Q91)/D(L93 E166), R23K,A113V	Este constructo contiene una forma híbrida de IFNaA y IFNaD fusionada corriente debajo de HSA madura.	pSAC35	174	142	206	243	244	HSA/kex2
27	1757	pSAC35 IL2.A21 TI53 145C/S.HSA	IL-2 humana madura con una mutación aminoácida simple (C a S en la posición 145) clonada corriente abajo de la secuencia líder HSA/KEX2 y corriente arriba de HSA madura.	pSAC35	175	143	207			HSA/kex2
28	1758	pSAC35 HSA.IL2 A21 - TI53 145C/S	IL-2 humana madura con una mutación aminoácido simple (C a S en la posición 145) clonada corriente debajo de HSA	pSAC35	176	144	208			HSA/kex2

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			con una secuencia líder HSA/kex2							
29	1812	pSAC35 IL 2 A21-T153 HSA	Aminoácidos A21 a T153 de IL-2 fusionada corriente abajo de la secuencia líder HSA/kex2 y corriente arriba de HSA madura.	pSAC35	177	145	209			HAS/kex2
30	1813	pSAC35 H SA.IL2.1 1 T153	Aminoácidos A21 a T153 de IL-2 fusionada corriente abajo de HAS con una secuencia líder HSA/kex2.	pSAC35	178	146	210			HAS/kex2
31	1952	pcDNA3 1 I L2.HSA	IL-2 humana de longitude completa, con una mutación de Cisteína a Serina en el aminoácido 145, fusionada corriente arriba de HSA madura.	pCDNA3 1	179	147	211			Secuencia guía IL-2 nativa
32	1954	pC4 IL2 H SA	IL-2 humana de longitud completa, con una mutación de Cisteína a Serina en el aminoácido 145, fusionada corriente arriba de HSA madura.	pC4	180	148	212			Secuencia guía IL-2 nativa
33	3468	pSAC35 K Tsp.HSA.G H	Péptido señal de tóxina asesina seguido por HSA madura seguido por hormona de crecimiento.	pSAC35	313	245	381	449	450	
34	3469	pSAC35 In vsp.HSA.G H	Péptido señal de invertasa seguido por HSA madura seguido por hormona de crecimiento.	pSAC35	314	246	382	451	452	Invertasa
35	3470	pSAC35 A Psp.HSA.G H	Péptido señal de fosfatasa ácida seguido por HSA madura seguido por hormona de crecimiento.	pSAC35	315	247	383	453	454	Fosfatasa acida
36	3475	pSAC35 G 19Rsp.HSA .HG	Secuencia de señal HSA/kex2 modificada seguida por HSA madura	pSAC35	316	248	384	455	456	HAS/kex2 modificada

			seguida por hormona de crecimiento.							
37	3476	pSAC35 G 19Rsp HSA IFNa	La secuencia de señal HSA/kex2 modificada seguida por HSA madura seguida por INF-alfa.	pSAC35	317	249	385	457	458	HAS/kex2 modificada
38	3549	pSAC35 H SA/kex2.B DNFc.HSA	La secuencia líder HSA/kex2 seguida por BDNfc madura (variante de empalme 6) seguida por HSA madura.	pSAC35	318	250	386	Ninguna	Ninguna	HSA/kex2
39	3610	pSAC35® HSA/KEX(R19G))SP GLP 1(7 36AG)x2 H SA	Secuencia líder HSA/kex2 modificada seguida por codon de levadura optimizado glp-1 dímero (donde el segundo dímero ha sido alterado de manera que la posición de fluctuación de cada codon es diferente del primer dímero) fusionado al extremo N de HSA	pSAC35	319	251	387	459	460	HSA/kex2 modificada
40	3690	pC4 MPIF SP BNP/HS A	Secuencia de señal de factor-1 inhibitorio progenitor mieloide (MPIF) seguida por BNP fusionada al extremo N de HSA madura.	PC4	320	252	388	461	462	MPIF 1
41	3691	pC4 SPCO N BNP/HS A	Una secuencia de señal de consenso seguida por BNP fusionada al extremo N de HSA madura.	pC4	321	253	389	463	464	Consenso
42	3696	pEE12 1 G LP 1(7 36(A8G)x2 HSA	Secuencia de señal de factor-1 inhibitorio progenitor mieloide (MPIF) seguida por dímero glp-1 optimizado de codon de levadura (donde el segundo dímero ha sido alterado de	pEE12 1	322	254	390	465	466	MPIF 1

			manera que la posición de fluctuación de cada codon es diferente del primer dímero) fusionada al extremo-N de HSA.							
43	3715	pSAC35 BNP29/HAS.S65	Una copia simple de BNP humana (aminoácidos 1 29) fusionada al extremo N- de HSA (S65-L585), una truncación N terminal de HSA (delta 1-64). Esta se encuentra corriente abajo de la secuencia de señal HSA/Kex2	pSAC35	323	255	391	467	468	HSA/kex2
44	3723	pEE12 1M PIFSP BNP /HSA	Secuencia de señal de factor-1 inhibitorio progenitor mieloide (MPIF) seguida por BNP fusionada al extremo-N de HSA madura.	pEE12 1	324	256	392	Ninguna	Ninguna	MPIF 1
45	3724	pEE12 1 2P CON BNP/ HSA	Una secuencia de señal de consenso seguida por BNP fusionada al extremo-N de HSA madura	pEE12 1	325	257	393	Ningunas	Ninguna	Consensos
46	3725	pEE12 1 SP CON2.BNP /HSA	Una secuencia de señal de consenso seguida por BNP fusionada al extremo-N de HSA madura.	PEE12 1	326	258	394	Ninguna	Ninguna	
47	3736	pC4 SPCO N2 BNP/HS A	Una secuencia de señal de consenso seguida por BNP fusionada al extremo-N de HSA madura.	pC4	327	259	395	469	470	
48	3769	pC4 BNP(R13G)/HS A	Secuencia de señal de factor-1 inhibitorio progenitor mieloide (MPIF)	pC4	328	260	396	471	472	MPIF 1
49	'3778	pC4 SPCO N BNP29/H AS.S65	Una copia simple de BNP humana (1 29) fusionada al extremo-N de HSA (S65-L585), una	pC34	329	261	397	473	474	Consensos

			truncación N-terminal de HSA (delta 1-64). Esta se encuentra corriente abajo de una secuencia de señal de consenso.							
50	3795	pC4 SPCON BNP(R13G)/HSA	Una secuencia de señal de consenso seguida por mutante de BNP (R13G) fusionada al extremo N de HSA madura.	pC4	330	262	398	475	476	Consenso
51	3795	pC4 SPCON BNP(K14G)/HSA	Secuencia de señal de consenso seguida por BNP mutante (R13G) fusionada al extremo-N de HSA madura.	pC4	331	263	399	477	478	Consenso
52	3796	pSAC35 HAS/BNP	Secuencia de señal de consenso seguida por BNP mutante (K14G) fusionada al extremo-N de HSA madura	pSAC35	332	264	400	479	480	HSA/Kex2
53	3809	pSAC35 BNP30(GGG)/HSA	Seguida por HSA madura fusionada al extremo N de BNP	pSAC35	333	265	401	481	482	HSA/Kex2
54	3886	pSAC35 HSA/KEX2 LANP HSA	Secuencia de señal HSA/kex2 seguida por BNP (aminoácidos 1-30) fusionada a través de glicinas tripartitas al extremo-N de HSA madura	pSAC35	334	266	402	Ninguna	Ninguna	HSA/Kex2
55	3887	pSAC35HSA/KEX2 HSA.LANP	Secuencia de señal HSA/kex2 seguida por LANP fusionada al extremo-N de HSA madura. LANP corresponde a aminoácidos 26-55 de SeqID No. 402 (conocida en la presente como LANP).	pSAC35	336	268	404	Ninguna	Ninguna	HSA/Kex2

56	3888	pSAC35 HSA/KEX2 VDP HSA	Secuencia de señal HSA/kex2 seguida por HSA madura fusionada al extremo N de LANP madura.	pSAC35	336	268	404	Ninguna	Ninguna	HSA/Kex2
57	3890	pSAC35 HSA/KEX2. KUP HSA	Secuencia de señal HSA/kex2 seguida por VDP fusionada al extremo N de HSA madura. VDP corresponde a aminoácidos 56-92 de SeqID No. 404 (conocida en la presente como VDP).	pSAC35	338	270	406	Ninguna	Ninguna	HSA/Kex2
58	3890	pSAC35HSA/KEX2 KUP HSA	Secuencia de señal HSA/kex2 seguida por HSA madura fusionada al extremo-N de VDP madura.	pSAC35	338	270	406	Ninguna	Ninguna	HSA/Kex2
59	3891	pSAC35 HSA/KEX2 HSA KUP	Secuencia de señal HSA/kex2 seguida por KUP fusionada al extremo N de HSA madura. KUP corresponde a los aminoácidos 104-123 de SeqID No. 406 (conocida en la presente como KUP).	pSAC35	339	271	407	Ninguna	Ninguna	HSA/Kex2
60	3892	pSAC35 HSA/KEX2. CNP HSA	Secuencia de señal HSA/kex2 seguida por HSA madura fusionada al extremo-N de KUP madura.	pSAC35	340	272	408	Ninguna	Ninguna	HSA/Kex2
61	3893	pSAC35 HSA/KEX2 HSA.CNP	Secuencia de señal HSA/kex2 seguida por CNP fusionada al extremo-N de HSA madura CNP corresponde a aminoácidos 105-123 de SeqID No. 408 (conocida en la presente como KUP)	pSAC35	341	273	409	Ninguna	Ninguna	HSA/Kex2
62	3894	pSAC35 H	Secuencia de señal	pSAC35	342	274	410	Ningun	Ningun	HSA/Kex2

		SA/KEX2 DNP HSA	HSA/Kex2 seguida por HSA madura fusionada al extremo-N de CNP madura					a	a	
63	3895	pSAC35 H SA/KEX2 HSA DNP	Secuencia de señal HSA/kex2 seguida por DNP fusionada al extremo-N de HSA madura	pSAC35	343	275	411	Ninguna	Ninguna	HSA/Kex2 modificada
64	3896	pSAC35 H SA/KEX(R 19G)-BNP30(GG G) HSA	Secuencia de señal HSA/kex2 seguida por HSA madura fusionada al extremo N de DNP madura.	pSAC35	344	276	412	Ninguna	Ninguna	HSA/Kex2 modificada
65	3897	pEE12 1 SP CON BNP(K14G).HSA	Secuencia líder HSA/kex-2 modificada seguida por BNP (aminoácidos 1-30) fusionada a través de enlazador de glicina tripartita al extremo-N de HSA madura.	pEE12 1	345	277	413	Ninguna	Ninguna	Consenso
66	3898	pSAC35 Ke x-2 BNP(K14G).HSA	Una secuencia de señal de consenso seguida por BNP mutante (K14G) fusionada al extremo-N de HSA madura.	pSAC35	346	278	414	Ninguna	Ninguna	HSA/Kex2
67	3899	pSAC35 H SA/Kex-2(R19G)-BNP(K14G).HSA	Secuencia de señal HSA/KEX-2 seguida por BNP mutante (K14G) fusionada al extremo N de HSA mutante.	pSAC35	347	279	415	Ninguna	Ninguna	HSA/Kex2 modificada
68	3900	pSAC35 hs a/kEX-2-hsa bnp(k1 4g)	Secuencia de señal HSA/KEX 2 modificada seguida por BNP mutante (K14G) fusonada al extremo N de HSA madura	pSAC35	348	280	416	Ninguna	Ninguna	HSA/Kex2
69	3903	PC4 SPCO N-uricase.HS	Secuencia de señal HSA/KEX 2 seguida por HSA madura fusionada al	pC4	349	281	417	Ninguna	Ninguna	Consenso

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		A	extremo-N de BNP mutante (K14G).							
70	3904	PC4 HAS/ Hex2-HAS.Uricas e	Una secuencia de señal de consenso seguida por uricasa de rata fusionada al extremo N de HSA madura	pC4	350	282	418	Ningun a	Ningun a	HSA/Kex2
71	3910	pSAC35 H SA/Kex-2-ADI.HSA	Secuencia de señal HSA/KEX-2 seguida por HSA madura fusionada al extremo-N de uricasa de rata	pSAC35	351	283	419	Ningun a	Ningun a	HSA/KeX2
72	3915	pC4 SPCO N ADI HSA	Secuencia de señal HSA/KEX-2 seguida por deiminasa de arginina de micoplasma fusionada al extremo-N de HSA madura.	pC4	352	284	420	Ningun a	Ningun a	Concensa
73	3917	PC4 HAS/ Kex 2 HAS.EDI	Una secuencia de señal de consenso seguida por deiminasa de arginina de micoplasma fusionada al – termino-N de HSA madura	pC4	353	285	421	Ningun a	Ningun a	HSA/Kex2
74	3918	pSAC35 H SA/Kex2-HSA ADI	Secuencia de señal HSA/KEX 2 seguida por HSA madura fusionada al extremo-N de deiminasa de arginina de micoplasma.	pSAC35	354	286	422	Ningun a	Ningun a	HSA/Kex2
75	3918	pSAC35 hs a/Kex2-HSA.ADI	Secuencia de señal HSA/KEX 2 seguida por HSA madura fusionada al extremo-N de deiminasa arginina de micoplasma.	pSAC35	355	287	423	Ningun a	Ningun a	HSA/Kex2
76	3920	pSAC35 H SA/Kex2-GCB.HSA	Secuencia de señal HSA/KEX 2 seguida por TweakR (aminoácidos 28-75) fusionados al extremo-N de HSA madura	pSAC35	356	288	424	Ningun a	Ningun a	HSA/Kex2

			TweakR E28-F75 corresponde a aminoácidos 28-75 de SeqID No. 423 (conocida en la presente como TweakR R28-F75).							
77	3921	PC4 SPCON-GCB.HSA	Secuencia de señal HSA/KEX 2 seguida por glucocerebrosidasa lisosomal fusionada al extremo-N de HSA madura.	pC4	357	289	425	Ninguna	Ninguna	Consenso
78	3922	pSAC35 hsa/kEX2 HSA.GCB	Secuencia de señal HSA/KEX2 seguida por HSA madura fusionada al extremo N de glucocerebrosidasa lisosomática	pSAC35	358	290	426	Ninguna	Ninguna	HAS/Kex2
79	3923	pC4 HAS/Kex2-HAS.GCB	Secuencia de señal HSA/KEX2 seguida por HSA madura fusionada al extremo N de glucocerebrosidasa lisosomática	pC4	359	291	427	Ninguna	Ninguna	HSA/kex2
80	3924	pSAC35 HSA/Kex2-ADM HSA	Secuencia de señal HSA/KEX2 seguida por adrenomedulina fusionada al extremo N de glucocerebrosidasa lisosomática	pSAC35	360	292	428	Ninguna	Ningunas	HAS/Kex2
81	3925	pC4 SPCON ADM HSA	Una secuencia de señal de consenso seguida por adrenomedulina fusionada al extremo N de HSA madura	pC4	361	293	429	Ninguna	Ninguna	Consenso
82	3926	pSAC35 HSA/Kex2-HSA.ADM	Secuencia de señal HSA/KEX2 seguida por HSA fusionada al extremo N de adrenomedulina.	pSAC35	362	294	430	Ninguna	Ninguna	HAS/Kex2
83	3927	pC4 HSA	Secuencia de señal	pC4	363	295	431	Ningun	Ningun	HSA/Kex2

		Kex2-HSA.ADM	HSA/KEX-2 seguida por HSA fusionada al extremo N de adrenomedulina.					a	a	
84	3928	PC4 HSA. Kiss.E20-Q145	La región pre-pro de la secuencia de señal HSA seguida por HSA madura fusionada al extremo N de Kiss (aminoácidos 20-145). Kiss E20-Q145 corresponde a los aminoácidos 20-145 de SeqID No. 432 (citada en la presente como Kiss E20-Q145).	pC4	364	296	432	Ningun a	Ningun a	HSA pre-pro
85	3929	pSAC35 K EX2 HSA. KISS-1 E20-Q145	Secuencia de señal HSA/KEX-2 seguida por HSA madura fusionada al extremo N de Kiss (aminoácidos 20-145).	pSAC35	365	297	433	Ningun a	Ningun a	HAS/Kex2
86	3930	pSAC35 K EX2 Ephri nB1 K30-D229 HSA	Secuencia de señal HAS/Kex 2 seguida por Efrina B1 (aminoácidos 30-229) fusionada al extremo N de HSA madura La Efrina B1 K30-D229 corresponde a los aminoácidos 30-229 de SeqID No. 434 (citada en la presente como Efrina B1 K30-D229).	pSAC35	366	298	434	Ningun a	Ningun a	HAS/Kex2
87	3931	PC4 Ephiri nB1 M1-D229 HSA	Efrina B1 (aminoácidos 1 229), incluyendo la secuencia de señal de Efrina B1 nativa, fusionada al extremo N de HSA madura.	pC4	367	299	435	Ningun a	Ningun a	Epirin B1 nativa
88	3932	PC4 TWE AKR.M1 F75.HSA	TweakR (aminoácidos 1-75), incluyendo la secuencia de señal TweakR nativa, fusionada	pC4	368	300	436	Ningun a	Ningun a	T wearkR native

			al extremo N de HSA madura TweakR M1 F75 corresponde a los aminoácidos 1 75 de SeqID No 436 (conocida en la presente como TweakR M1 F75).							
89	3933	PC4 B7 H3.M1 E247 HSA	B7 H3 (aminoácidos 1-247), incluyendo la secuencia de señal B7-H3 nativa, fusionada al extremo N de HSA madura.	pC4	369	301	437	Ninguna	Ninguna	B7 H3 nativa
90	3934	PC4 HAS. EphrinB1 K30-D229	La pre-pro-región de la secuencia de señal de HAS seguida por HAS madura fusionada al extremo N de Efrina B1 (aminoácidos 30-229).	pC4	370	302	438	Ninguna	Ninguna	HAS pre-pro
91	3935	pSAC35 HSA EphrinB1 K30-D229	La secuencia de señal HAS/Kex2 seguida por HAS madura fusionada al extremo N de Efrina B1 (aminoácidos 20-229).	pSAC35	371	303	439	Ninguna	Ninguna	HAS/Kex2
92	3936	PC4 SPCON cUricase. HSA	Una secuencia de señal de consenso seguida por uricasa de chimpance fusionada al extremo N de HSA madura.	pC4	372	304	440	Ninguna	Ninguna	Consensus
93	3937	pC4 HAS.cUricase	La pre-pro-región de la secuencia de señal HAS seguida por HSA madura fusionada al extremo N de uricasa de chimpance.	pC4	373	305	441	Ninguna	Ninguna	HAS pre-pro
94	3938	PC4 SPCON hUricase. HSA	Una secuencia de señal de consenso seguida por uricasa fusionada al extremo N de HAS madura	pC4	374	306	442	Ninguna	Ninguna	Consenso
95	3939	PC4 HAS.h	La pre-pro-región de la	pC4	375	307	443	Ningun	Ningun	HAS pre-pro

		Uricase	secuencia de señal HSA seguida por HSA madura fusionada al extremo N de uricasa.					a	a	
96	3940	PC4 SPCO N bUricase. HSA	Una secuencia de señal de consenso seguida por uricasa de mandril fusionada al extremo N de HAS madura.	pC4	376	308	444	Ningun a	Ningun a	Consenso
97	3941	PC4 HAS.b Uricase	La pre-pro-región de la secuencia de señal HSA seguida por HSA madura fusionada al extremo N de uricasa de mandril.	pC4	377	309	445	Ningun a	Ningun a	HAS pre-pro
98	3942	pSAC35 K EX2 bUrica se HSA	La secuencia de señal HSA/Kex2 seguida por uricasa fusionada al extremo N de HSA madura	pSAC35	378	310	446	Ningun a	Ningun a	HAS/Kex2
99	3943	pSAC35 K EX2 cUrica se HSA	La secuencia de señal HSA/Kex2 seguida por uricasa de chimpancé fusionada al extremo N de HSA madura.	pSAC35	379	311	447	Ningun a	Ningun a	HAS/Kex2
100	3944	pSAC35 K EX2 bUrica se.HSA	La secuencia de señal HSA/Kex2 seguida por uricasa de mandril fusionada al extremo N de HSA madura.	pSAC35	380	312	448	Ningun a	Ningun a	HAS/kex2
101	3618	PC4 SPCO N BNPI-32(2x)/HAS	Una secuencia de señal de consenso siguida por dos copias en tandem de BNP madura fusionada al extremo N de HSA madura.	PC4	483	484	485	463	464	consenso

La tabla 2 proporciona una lista no exhaustiva de polinucleótidos del invento comprendiendo o alternativamente constando de moléculas de ácido nucleico que codifican una proteína de fusión de albúmina. La primera columna "Fusión No." Proporciona un número de fusión a cada polinucleótido. La columna 2, "Constructo ID" proporciona un identificador numérico único para cada polinucleótido del invento. Los IDs de constructor se pueden utilizar para identificar polinucleótidos que codifican proteínas de fusión de albúmina comprendiendo o alternativamente constando de una porción de proteína Terapéutica que corresponde a una proteína Terapéutica dada: X listada en la correspondiente fila de la Tabla 1 en donde el ID del constructo se lista en la columna 5. La columna "Nombre de Constructo" (columna 3) proporciona el nombre de un polinucleótido o constructo de fusión de albúmina dado.

La cuarta columna en la tabla 2, "Descripción" proporciona una descripción general de un constructo de fusión de albúmina dado, y la quinta columna, "vector de expresión" lista el vector en el cual un Polinucleótido que comprende o alternativamente consta de una molécula de ácido nucleico que codifica una proteína de fusión de albúmina dada, fue clonado. Los vectores son conocidos en el estado de la técnica y están disponibles comercialmente o se describen en otras partes. Por ejemplo, como se describe en los ejemplos, un "cassete de expresión" que comprende o alternativamente consta de uno o más entre (1) un Polinucleótido que codifica una proteína de fusión de albúmina dada, (2) una secuencia guía, (3) una región promotora, y (4) un terminador transcripcional, se puede ensamblar en un vector de clonación conveniente y subsecuentemente mover hacia un vector alternativo, tal como, por ejemplo un vector de expresión incluyendo, por ejemplo, un vector de expresión de levadura o un vector de expresión mamífero. En una realización, para expresión en *S. cervisae,* un casete de expresión que comprende, o alternativamente consta de una molécula de ácido nucleico que codifica una proteína de fusión de albúmina es clonado en pSAC35. En otra realización, para expresión en células CHO, un casete de expresión que comprende, o alternativamente consta de una molécula de ácido nucleico que codifica una proteína de fusión de albúmina es clonado en pC4. En una realización adicional, un Polinucleótido que comprende o alternativamente consta de una molécula de ácido nucleico que codifica la porción de proteína Terapéutica de una proteína de fusión de albúmina es clonado en pC4:HSA. En aún una realización adicional, para expresión en células NS0, un casete de expresión que comprende o alternativamente consta de una molécula de ácido nucleico que codifica una proteína de fusión de albúmina es clonado en pEE12. Otros vectores de clonación y/o expresión útiles serán conocidos por alguien dentro del estado de la técnica y están dentro del alcance del invento.

La columna 6, "SEQ ID NO:Y," proporciona la secuencia de aminoácidos de longitud total de la proteína de fusión de albúmina del invento. En la mayoría de instancias, SEQ ID NO:Y muestra la forma no procesada de la proteína de fusión de albúmina codificada –en otras palabras, SEQ ID: NO: Y muestra la secuencia de señal, una porción HSA, y una porción terapéutica todas codificadas por el constructo particular. Específicamente contemplados por el presente invento están todos los polinucleótidos que codifican SEQ ID NO:Y. Cuando estos polinucleótidos se utilizan para expresar la proteína codificada a partir de una célula, la secreción natural de la célula y pasos de procesamiento produce una proteína que carece de la secuencia de señal listada en las columnas 4 y/o 11 de la tabla 2. La secuencia de aminoácidos especifica de la secuencia de señal listada es mostrada luego en la especificación o es bien conocida en el estado de la técnica. Así, las realizaciones más preferidas del presente invento incluyen la proteína de fusión de albúmina producida por una célula (la cual carecería de la secuencia guía mostrada en las columnas 4 y/o 11 de la Tabla 2). También preferidos son los polipéptidos que comprenden la

SEQ ID NO:Y sin la secuencia guía especifica listada en las columnas 4 y/o 11 de la Tabla 2. También se prefieren composiciones que comprenden estas dos realizaciones preferidas, incluyendo composiciones farmacéuticas. Además, está dentro de la capacidad de alguien con experiencia en el estado de la técnica, reemplazar la secuencia de señal listada en las columnas 4 y/o 11 de la Tabla 2 con una secuencia de señal diferentes, tal como aquellas descritas luego en la especificación para facilitar la secreción de la proteína de fusión de albúmina procesada.

La séptima columna, "SEQ ID NO:X," proporciona la secuencia precursora de ácido nucleico a partir de la cual se ha derivado un polinucleótido que codifica una porción de proteína terapéutica de una proteína de fusión de albúmina dada. En una realización, la secuencia precursora de ácido nucleico a partir de la cual se puede derivar un Polinucleótido que codifica una porción de proteína Terapéutica de una proteína de fusión de albúmina comprende la secuencia genética de tipo silvestre que codifica una proteína Terapéutica mostrada en la Tabla 1. En una realización alternativa, la secuencia precursora de ácido nucleico a partir de la cual se puede derivar un Polinucleótido que codifica una porción de proteína Terapéutica de una proteína de fusión de albúmina, comprende una variante o derivado de una secuencia genética de tipo silvestre que codifica una proteína Terapéutica mostrada en la Tabla 1, tal como por ejemplo, un variante optimizado de codón sintético de una secuencia genética de tipo silvestre que codifica una proteína Terapéutica.

La columna octava, "SEQ ID NO:Z," proporciona una traslación predicha de la secuencia precursora de ácido nucleico (SEQ ID NO:X). Esta secuencia precursora puede ser una proteína precursora de longitud completa utilizada para derivar el constructo particular, la porción madura de una proteína precursora, una variante o fragmento de una proteína tipo silvestre, o una secuencia artificial que se puede utilizar para crear el constructo descrito. Alguien con experiencia en el estado de la técnica puede utilizar esta secuencia de aminoácidos mostrada en SEQ ID NO:Z para determinar cuales residuos aminoácidos de una proteína de fusión de albúmina codificada por un constructo dado son proporcionados por la proteína Terapéutica. Además, está dentro de la capacidad de alguien con experiencia, utilizar la secuencia mostrada en SEQ ID NO:Z para derivar el constructo descrito en la misma fila. Por ejemplo, si SEQ ID NO:Z corresponde a una proteína de longitud completa, pero solamente una porción de esa proteína se utiliza para general el CID especifico, está dentro de la destreza del estado de la técnica confiar en técnicas de biología molecular, tales como PCR, para amplificar el fragmento específico y clonarlo en el vector apropiado.

Los cebadores de amplificación proporcionados en las columnas 9 y 10, "SEQ ID NO:A" y "SEQ ID NO:B" respectivamente, son cebadores de ejemplo utilizados para generar un polinucleótido que comprende o alternativamente consta de una molécula de ácido nucleico que codifica la porción de proteína Terapéutica de una proteína de fusión de albúmina dada. En una realización del invento, los cebadores oligonucleotidos que tienen las secuencias mostradas en las columnas 9 y/o 10 (SEQ ID NO: A y/o B) se utilizan para amplificar PCR de un Polinucleótido que codifica la porción de proteína Terapéutica de una proteína de fusión de albúmina utilizando una molécula de ácido nucleico que comprende o alternativamente consta de la secuencia nucleótida proporcionada en la columna 7 (SEA ID NO:X) de la correspondiente fila como el ADN plantilla. Los métodos PCR son bien establecidos en el estado de la técnica. Secuencias cebadoras útiles adicionales podrían fácilmente ser modificadas y utilizadas por aquellos con experiencia común en el estado de la técnica.

En una realización alternativa, los cebadores oligonucleótidos se pueden utilizar en reacciones PCR translapadas para generar mutaciones dentro de una secuencia ADN plantilla. Los métodos PCR son bien conocidos dentro del estado de la técnica.

Como se muestra en la Tabla 3, ciertos constructor de fusión de albúmina divulgados en esta solicitud se han depositado con ATCC®

Tabla 3

ID del Constructo	Nombre del Constructo	Numero de deposito ATCC No./ Fecha
1812	pSAC35:IL2.A21-T153.HSA	PTA-3759 Oct. 4, 2001
2053	pEE12:IFNb-HSA también denominado pEE12.1IFβ-HSA	PTA-3764 Oct. 4, 2001
2054	pEE12:HSA-IFNb	PTA-3941 Dec. 19, 2001
2249	pSAC35:IFNa2-HSA tambien denominado pSAC23:IFNα2-HSA	PTA-3763 Oct. 4, 2001
2343	pSAC35.INV-IFNA2.HSA	PTA-3940 Dec. 19,2001
2381	pC4:HSAIFNa2(C17-E181)	PTA-3942 Dec. 19, 2001
2382	pC4:IFNa2-HSA	PTA-3939
2492	pC4.IFNb(delta M22).HSA	PTA-3943 Dec. 19,2001
3165	pSAC35:HSA.IFNa también denominado CID 3165, pSAC35:HSA.INFα	PTA-4670 Sept. 16, 2002

Es posible recuperar un constructo de fusión de albúmina dado a partir del depósito mediante técnicas conocidas en el estado de la técnica y descritas en la presente (*ver*, Ejemplo 10). El ATCC está localizado en 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. Los depósitos ATCC fueron hechos conforme al Tratado de Budapest sobre el reconocimiento internacional del depósito de microorganismos para propósitos de procedimiento de patentes.

En una realización adicional del invento, un "casete de expresión" que comprende o alternativamente consta de uno o más entre (1) un polinucleótido que codifica una proteína de fusión de albúmina dada, (2) una secuencia guía, (3) una región promotora, y (4) un terminador transcripcional, se puede mover o "subclonar" de un vector a otro. Los fragmentos a ser subclonados se pueden generar mediante métodos bien conocidos en el estado de la técnica, tales como, por ejemplo, amplificación PCR (ej., utilizando cebadores oligonucleótidos con la secuencia mostrada en SEQ ID NO: A o B), y/o digestión de enzimas de restricción.

En realizaciones preferidas, las proteínas de fusión de albúmina del invento son capaces de una actividad terapéutica y/o actividad biológica correspondiente a la actividad terapéutica y/o actividad biológica de la proteína Terapéutica correspondiente a la porción de proteína Terapéutica de la proteína de fusión de albúmina listada en la correspondiente fila de la Tabla 1. En realizaciones preferidas adicionales, las porciones de proteínas terapéuticamente activas de

proteínas de fusión de albúmina del invento son fragmentos o variantes de la proteína codificada por la secuencia mostrada en la columna SEQ ID NO:X de la Tabla 2, y son capaces de la actividad terapéutica y/o actividad biológica de la proteína Terapéutica Correspondiente.

Fragmentos y Variantes de Polipéptidos y Polinucleótidos

Fragmentos

El presente invento está dirigido adicionalmente a las proteínas Terapéuticas descritas en la Tabla 1, proteínas de albúmina, y/o proteínas de fusión de albúmina del invento.

El presente invento también está dirigido a polinucleótidos que codifican fragmentos de las proteínas Terapéuticas descritas en la Tabla 1, proteínas de albúmina, y/o proteínas de fusión de albúmina del invento.

Aún si la eliminación de uno o más aminoácidos a partir del N-término de una proteína resulta en modificación o perdida de una o más funciones biológicas de la proteína Terapéutica, proteína de albúmina, y/o proteína de fusión de albúmina del invento, otras actividades terapéuticas y/o actividades funcionales (ej., actividades biológicas, capacidad para multimerizar, capacidad para unir un ligando) pueden aún ser retenidas. Por ejemplo, la capacidad de los polipéptidos con eliminaciones por el extremo N-terminal para inducir y/o unirse a anticuerpos los cuales reconocen las formas completas y maduras de los polipéptidos, generalmente será retenida cuando menos de la mayoría de los residuos del polipéptido completo son retirados del N-termino. Se puede determinar fácilmente mediante métodos comunes descritos en la presente o de otra manera conocidos en el estado de la técnica, si un polipéptido particular que carece de residuos N-terminales de un polipéptido completo retiene tales actividades inmunológicas. No es improbable que una muteina (proteína resultante de una mutación) con un gran número de residuos aminoácidos N-terminales eliminados pueda retener algunas actividades biológicas o inmunogénicas. En realidad, péptidos compuestos de tan solo seis residuos aminoácidos pueden a menudo evocar una respuesta inmune.

Por consiguiente, fragmentos de una proteína Terapéutica correspondiente a una porción de proteína Terapéutica de una proteína de fusión de albúmina del invento, incluyen la proteína de longitud total así como también polipéptidos con uno o más residuos eliminados desde el termino amino de la secuencia de aminoácidos del polipéptido de referencia (es decir, una proteína Terapéutica citada en la Tabla 1, o una porción de proteína Terapéutica de una proteína de fusión de albúmina codificada por un Polinucleótido o constructo de fusión de albúmina descrito en la Tabla 2). En particular, las eliminaciones por el extremo N-terminal se pueden describir mediante la fórmula general m a q, donde q es un entero que representa el número total de residuos aminoácidos en un péptido de referencia (ej., una proteína Terapéutica citada en la Tabla 1, o una porción de proteína terapéutica de una proteína de fusión de albúmina del invento, o una porción de proteína Terapéutica de una proteína de fusión de albúmina codificada por un Polinucleótido o constructo de fusión de albúmina descrito en la Tabla 2), y m se define como cualquier entero en el rango entre 2 y q menos 6. Los polinucleótidos que codifican estos polipéptidos también son contemplados por el invento.

Además, los fragmentos de polipéptidos de albúmina sérica correspondientes a una porción de proteína de albúmina de una proteína de fusión de albúmina del invento, incluyen la proteína de longitud total así como también polipéptidos con uno o más residuos eliminados del término amino de la secuencia de aminoácidos del polipéptido de referencia (es decir, albúmina sérica, o una porción de albúmina sérica de una proteína de fusión de albúmina codificada mediante un Polinucleótido o constructo de fusión de albúmina descrito en la Tabla 2). En realizaciones preferidas, las eliminaciones por el extremo N-terminal se pueden describir mediante la fórmula general m a 585, donde 585 es un entero que representa el número total de

residuos aminoácidos en albúmina sérica humana madura (SEQ ID NO:1), y m se define como cualquier entero entre 2 y 579. Los polinucleótidos que codifican estos polipéptidos también son contemplados por el invento. En realizaciones adicionales, las eliminaciones por el extremo N-terminal se pueden describir mediante la fórmula general m a 609, donde 609 es un entero que representa el numero total de residuos aminoácidos en albúmina sérica humana de longitud total (SEQ ID NO:3), y m se define como cualquier entero entre 2 y 603. Los polinucleótidos que codifican estos polipéptidos también son contemplados por el invento.

Además, los fragmentos de proteínas de fusión de albúmina del invento, incluyen la proteína de fusión de albúmina de longitud completa así como también polipéptidos con uno o más residuos eliminados desde el termino amino de la proteína de fusión de albúmina (ej., una proteína de fusión de albúmina codificada por un Polinucleótido o constructo de fusión de albúmina descrito en la Tabla 2; o una proteína de fusión de albúmina con la secuencia aminoácido divulgada en la columna 6 de la Tabla 2). En particular, las eliminaciones por el extremo N-terminal se pueden describir mediante la fórmula general m a q, donde q es un entero que representa el numero total de residuos aminoácidos en la proteína de fusión de albúmina, y m se define como cualquier entero entre 2 y q menos 6. Los polinucleótidos que codifican estos polipéptidos también son contemplados por el invento.

También como se mencionó anteriormente, aún si la eliminación de uno o más aminoácidos desde el termino N o término C de un polipéptido de referencia (ej., una proteína terapéutica, proteína de albúmina sérica; o proteína de fusión de albúmina del invento) resulta en modificación o pérdida de una o mas funciones biológicas de la proteína, otras actividades funcionales (ej., actividades biológicas, capacidad para multimerizar, capacidad para unir a un ligando) y/o actividades Terapéuticas pueden aún ser retenidas. Por ejemplo la capacidad de los polipéptidos con eliminaciones C-terminales para inducir y/o unirse a anticuerpos que reconocen las formas completas o maduras del polipéptido generalmente será retenida cuando menos de la mayoría de los residuos del polipéptido completo o maduro son removidos del término C. Se puede determinar fácilmente mediante métodos comunes descritos en la presente o de otra manera conocidos en el estado de la técnica, si un polipéptido particular que carece de residuos N-terminales y/o C-terminales de un polipéptido de referencia retiene actividad Terapéutica.

El presente invento además proporciona polipéptidos con uno o mas residuos eliminados desde el término carboxilo de la secuencia de aminoácidos de una proteína Terapéutica correspondiente a una porción de proteína Terapéutica de una proteína de fusión de albúmina del invento (ej., una proteína Terapéutica citada en la Tabla 1, o una porción de proteína Terapéutica de una proteína de fusión de albúmina codificada por un Polinucleótido o constructo de fusión de albúmina descrito en la Tabla 2). En particular, las eliminaciones C-terminales se pueden describir por la fórmula general 1 a n, donde n es cualquier entero entre 6 y q menos 1, y donde q es un entero que representa el numero total de residuos aminoácidos en un polipéptido de referencia (ej., una proteína Terapéutica citada en la Tabla 1, o una porción de proteína Terapéutica de una proteína de fusión de albúmina codificada por un Polinucleótido o constructo de fusión de albúmina descrito en la Tabla 2). Los polinucleótidos que codifican estos polipéptidos también son contemplados por el invento.

Además, el presente invento proporciona polipéptidos con uno o más residuos eliminados desde el termino carboxilo de la secuencian de aminoácidos de una proteína de albúmina correspondiente a una porción de proteína de albúmina de una proteína de fusión de albúmina del invento (ej., albúmina sérica o una porción de proteína de albúmina de una proteína de fusión de albúmina codificada por un Polinucleótido o constructo de fusión de albúmina descrito en la Tabla 2). En particular, las eliminaciones C-terminales se pueden describir mediante la fórmula

general 1 a n, donde n es cualquier entero entre 6 y 584, donde 584 es el entero completo que representa el numero total de residuos aminoácidos en albúmina sérica humana madura (SEQ ID: 1) menos 1. Los polinucleótidos que codifican estos polipéptidos también son contemplados por el invento. En particular, las eliminaciones c-terminales se pueden describir mediante la fórmula general 1 a n, donde n es cualquier entero entre 6 y 608, donde 608 es el entero completo que representa el número total de residuos aminoácidos en albúmina sérica (SEQ ID:NO:3) menos 1. Los polinucleótidos que codifican estos polipéptidos también son contemplados por el invento.

Además, el presente invento proporciona polipéptidos con uno más residuos eliminados desde el término carboxilo de una proteína de fusión de albúmina del invento. En particular, las eliminaciones C-terminales se pueden describir mediante la fórmula general 1 a n, donde n es cualquier entero completo entre 6 y q menos 1, y donde q es un entero que representa el número total de residuos aminoácidos en una proteína de fusión de albúmina del invento. Los polinucleótidos que codifican estos polipéptidos también son contemplados por el invento.

Además, cualquiera de las eliminaciones N- o C-terminales anteriormente descritas se pueden combinar para producir un polipéptido de referencia N- y C-terminal suprimido. El invento también proporciona polipéptidos con uno o más aminoácidos eliminados desde tanto el término amino como el término carboxilo, los cuales se pueden describir generalmente por tener residuos m a n de un polipéptido de referencia (ej., una proteína terapéutica citada en la Tabla 1, o una porción de proteína Terapéutica de una proteína de fusión de albúmina del invento, o una porción de proteína Terapéutica codificada mediante un Polinucleótido o constructo de fusión de albúmina descrito en la Tabla 2, o albúmina sérica (ej., SEQ ID NO:1), o una porción de proteína de albúmina de una proteína de fusión de albúmina del invento, o una porción de proteína de albúmina codificada por un Polinucleótido o constructo de fusión de albúmina descrito en la Tabla 2, o una proteína de fusión de albúmina, o una proteína de fusión de albúmina codificada por un Polinucleótido o constructo de fusión de albúmina del invento) donde n y m son enteros como se describió anteriormente. Los polinucleótidos que codifican estos polipéptidos también son contemplados por el invento.

La presente solicitud también está dirigida a proteínas que contienen polipéptidos al menos 80%, 90%, 95%, 96%, 97%, 98% o 99% idénticos a una secuencia polipéptida de referencia (ej., una Proteína Terapéutica citada en la Tabla 1, o una porción de Proteína Terapéutica de una proteína de fusión de albúmina del invento, o una porción de Proteína Terapéutica codificada por un Polinucleótido o constructo de fusión de albúmina descrito en la Tabla 2, o albúmina sérica (ej., SEQ ID NO: 1), o una porción de proteína de albúmina de una proteína de fusión de albúmina del invento, o porción de proteína de albúmina codificada por un Polinucleótido o un constructo de fusión de albúmina descrito en la tabla 2, o una proteína de fusión de albúmina, o una proteína de fusión de albúmina codificada mediante un Polinucleótido o constructo de fusión de albúmina del invento) divulgada en la presente, o fragmentos de la misma. En realizaciones preferidas, la solicitud está dirigida a proteínas que comprenden polipéptidos al menos 80%, 90%, 95%, 96%, 97%, 98% o 99% idénticos a una secuencia polipéptida de referencia con la secuencia de aminoácidos de eliminaciones N- y C-terminales como se describió anteriormente. Los polinucleótidos que codifican estos polipéptidos también son contemplados por el invento.

Los fragmentos de polipéptidos preferidos del invento son fragmentos que comprenden o alternativamente consta de una secuencia de aminoácidos que presenta una actividad Terapéutica y/o funcional (ej., actividad biológica) de la secuencia polipéptida de la proteína Terapéutica o proteína de albúmina sérica de la cual la secuencia de aminoácidos es un fragmento.

Otros fragmentos polipéptidos preferidos son fragmentos biológicamente activos. Los fragmentos biológicamente activos son aquellos que exhiben actividad similar, pero no necesariamente idéntica al polipéptido del presente invento. La actividad biológica de los fragmentos puede incluir una actividad deseada mejorada, o una actividad indeseada disminuida.

Variantes

"Variante" se refiere a un Polinucleótido o ácido nucleico que difiere de un polipéptido o ácido nucleico de referencia, pero reteniendo las propiedades esenciales del mismo. Generalmente, las variantes son muy similares, y, en muchas regiones, idénticas al polipéptido o ácido nucleico de referencia.

Como se utiliza en la presente, "variante", se refiere a una porción de proteína Terapéutica de una proteína de fusión de albúmina del invento, porción de albúmina de una proteína de fusión de albúmina del invento, o proteína de fusión de albúmina del invento que difiere en secuencia de una proteína Terapéutica (ej., ver columna "terapéutica" de la Tabla 1), proteína de albúmina, y/o proteína de fusión de albúmina, respectivamente, pero reteniendo al menos una propiedad funcional y/o terapéutica de la misma como se describe en la presente o de otra manera se conoce en el estado de la técnica. Generalmente, las variantes son muy similares, y en muchas regiones, son idénticas a la secuencia de aminoácidos de la proteína Terapéutica correspondiente a una porción de Proteína Terapéutica de una proteína de fusión de albúmina, proteína de albúmina que corresponde a porción de proteína de albúmina de una proteína de fusión de albúmina, y/o una proteína de fusión de albúmina. Los ácidos nucleicos que codifican estas variantes también son contemplados por el invento.

El presente invento también esta dirigido a proteínas las cuales comprenden o alternativamente constan de una secuencia aminoácido la cual es al menos 80%, 85%, 90%, 95%, 96%, 97%, 98% o 99% idéntica a, por ejemplo, la secuencia de aminoácidos de una proteína Terapéutica que corresponde a una porción de Proteína Terapéutica de una proteína de fusión de albúmina del invento (ej., la secuencia de aminoácidos de una proteína Terapéutica: X divulgada en la tabla 1; o la secuencia de aminoácidos de una porción de Proteína Terapéutica de una proteína de fusión de albúmina codificada por un Polinucleótido o constructo de fusión de albúmina descrito en la Tabla 1 y 2, o fragmentos o variantes del mismo), proteínas de albúmina correspondientes a porción de proteína de albúmina de una proteína de fusión de albúmina del invento (ej., la secuencia de aminoácidos de porción de proteína de albúmina de una proteína de fusión de albúmina codificada por un Polinucleótido o constructo de fusión de albúmina descrito en la Tabla 1 y 2; la secuencia de aminoácidos mostrada en SEQ ID NO: 1; o fragmentos o variantes de la misma), y/o proteínas de fusión de albúmina. Fragmentos de estos polipéptidos también son proporcionados (ej., aquellos fragmentos descritos en la presente). Además los polipéptidos contemplados por el invento son polipéptidos codificados por polinucleótidos que hibridizan al complemento de una molécula de ácido nucleico que codifica una proteína de fusión de albúmina del invento bajo condiciones de hibridización severas (ej., hibridización con el fin de filtrar ADN fijado en cloruro de sodio 6X/citrato de sodio (SSC) a aproximadamente 45° Celsius, seguida por uno o más lavados en 2.2X SCC, 0.1% SDS a 50 – 65 grados Celsius), bajo condiciones altamente severas (ej., hibridización para filtrar ADN fijado en cloruro de sodio 6X/citrato de sodio (SSC) a aproximadamente 45°C, seguida por uno o mas lavados en 0.1X SSC, 0.2% SDS a 68°C aproximadamente), o bajo otras condiciones de hibridización severas las cuales son conocidas por aquellos con experiencia en el estado de la técnica (ver por ejemplo, Ausubel, F.M. eds., 1989 *Current protocol in Molecular Biology,* Green publishing associates, Inc., y John Wiley & Sons Inc., New York, paginas 6.3.1 – 6.3.6 y 2.10.3). Los polinucleótidos que codifican estas polipéptidos también con contemplados por el invento.

Por un polipéptido con una secuencia de aminoácidos al menos, por ejemplo, 95% "idéntica" a una secuencia de aminoácidos de consulta, se pretende que la secuencia de aminoácidos del polipéptido en cuestión sea idéntica a la secuencia de consulta con la excepción que la secuencia polipeptida en cuestión puede incluir hasta cinco alteraciones de aminoácidos por cada 100 aminoácidos de la secuencia de aminoácidos de consulta. En otras palabras, para obtener un polipéptido con una secuencia de aminoácidos al menos 95% idéntica a una secuencia de aminoácidos de consulta, se pueden insertar, suprimir o sustituir con otro aminoácido hasta 5% de los residuos aminoácidos en la secuencia en cuestión. Estas alteraciones de la secuencia de referencia pueden ocurrir en la posiciones de los términos amino y carboxilo de la secuencia de aminoácidos de referencia o cualquier otra parte entre aquellas posiciones terminales, intercaladas ya sea de manera individual entre residuos en la secuencia de referencia o en uno o más grupos contiguos dentro de la secuencia de referencia.

Como una materia practica, se puede determinar convencionalmente mediante el uso de programas para computador si cualquier polipéptido particular es al menos 80%, 85%, 90%, 95%, 96%, 97%, 98% o 99% idéntico a, por ejemplo, la secuencia de aminoácidos de una proteína de fusión de albúmina del invento o un fragmento de la misma (tal como una porción de Proteína Terapéutica de la proteína de fusión de albúmina o una porción de albúmina de la proteína de fusión de albúmina). Un método preferido para determinar la mejor correspondencia general entre una secuencia de consulta (una secuencia del presente invento) y una secuencia en cuestión, también conocida como alineación de secuencia global, se puede determinar utilizando el programa de computador FASTDB con base en el algoritmo Brutlag (Comp. App. Biosci. 6.237-245 (1990)). En una alineación de secuencias, las secuencias de consulta y en cuestión son ambas secuencias nucleótidas o secuencias de aminoácidos. El resultado de dicha alineación de secuencias global se expresa como porcentaje de identidad. Los parámetros preferidos utilizados en una alineación de aminoácidos FASTDB son: Matriz= PAM 0, k-tuple=2, sanción por no correspondencia=1, sanción por unificación =20, Longitud de grupo aleatorio=0, puntaje de corte=1, Tamaño de Ventana=longitud de secuencia, sanción por brecha=5, sanción por tamaño de brecha =0.05, tamaño de ventana=500 o la longitud de la secuencia en cuestión de aminoácidos, cualesquiera sea más corta.

Si la secuencia en cuestión es más corta que la secuencia de consulta debido a las eliminaciones N- o C-terminales, no por las eliminaciones internas, se debe hacer una corrección manual a los resultados. Esto se debe a que el programa FASTDB no es responsable de truncamientos N- y C-terminales de la secuencia en cuestión cuando se calcula el porcentaje de identidad global. Para secuencias en cuestión truncadas en los extremos N y C, con relación a la secuencia de consulta, el porcentaje de identidad es corregido mediante el calculo del numero de residuos de la secuencia de consulta que son N- y C-terminales de la secuencia en cuestión, los cuales no corresponden/están alineados con un residuo en cuestión correspondiente, como un porcentaje de las bases totales de la secuencia de consulta. La alineación/correspondencia de un residuo se determina mediante los resultados de la alineación de secuencia FASTDB. Luego este porcentaje se resta del porcentaje de identidad, calculado por el programa FASTDB anterior utilizando los parámetros específicos, para llegar a un puntaje final de porcentaje de identidad. Este puntaje final de porcentaje de identidad es el que se utiliza para propósitos del presente invento. Solamente residuos a los terminos N y C de la secuencia en cuestión, los cuales no corresponden o están alineados con la secuencia de consulta, se consideran para propósitos de ajustar de manera manual el puntaje de porcentaje de identidad. Es decir, solamente porciones de residuos de consulta por fuera de los residuos N y C-terminales más remotos de la secuencia en cuestión.

Por ejemplo, una secuencia en cuestión de 90 residuos aminoácidos es alineada con una secuencia de consulta de 100 residuos para determinar el porcentaje de identidad. La eliminación ocurre en el termino N de la secuencia en cuestión y por lo tanto, la alineación FASTDB no muestra correspondencia alineación de los primeros 10 residuos en el término N. Los 10 residuos no emparejados representan 10% de la secuencia (numero de residuos en los términos N y C no corresponden/número total de residuos en la secuencia de consulta) de manera que se resta 10% del puntaje de porcentaje de identidad calculado por el programa FASTDB. Si los 90 residuos restantes perfectamente correspondieron, el porcentaje final de identidad sería 90%. En otro ejemplo, una secuencia en cuestión de 90 residuos es comparada con una secuencia de consulta de 100 residuos. Esta vez las eliminaciones son eliminaciones internas, de manera que no hay residuos en los extremos N o C de la secuencia en cuestión los cuales no corresponden/están alineados con la secuencia de consulta. En este caso el porcentaje de identidad calculado mediante FASTDB no se corrige manualmente. Una vez más, solamente las porciones de residuos por fuera de los extremos N y C-terminales de la secuencia en cuestión, como se presentó en la alineación FASTDB, las cuales no corresponden/están alineadas con la secuencia de consulta son corregidas de manera manual. No se realizan otras correcciones manuales para propósitos del presente invento.

La variante usualmente tendrá al menos 75% de identidad de secuencia (preferiblemente al menos 80%, 90%, 95% o 99%) con una longitud de proteína Terapéutica o HA normal que es la misma longitud que la variante. La homología o identidad a nivel de secuencia nucleótida o de aminoácidos se determina mediante análisis BLAST (Herramienta de Búsqueda de Alineación Local Básica) utilizando el algoritmo utilizado por los programas blastp, blastn, blastx, tblastn y tblastx (karlin. Proc. Natl. Acad. Sci. USA 87: 2264-2268 (1990) y Altschul, J. Mol. Evol. 36: 290-300 (1993), incorporados completamente como referencia) los cuales son adaptados para búsqueda de similitud de secuencias.

El enfoque utilizado por el programa BLAST es primero considerar segmentos similares entre una secuencia de consulta y una secuencia de base de datos, luego evaluar el significado estadístico de todas las correspondencias que son identificadas y finalmente resumir solamente aquellas correspondencias que satisfacen un umbral de significado preseleccionado. Para una discusión de temas básicos en búsqueda de similitud de bases de datos de secuencias ver Altschul (Nature Genetics 6: 119-129 (1994)) el cual es incorporado completamente como referencia. Los parámetros de búsqueda para histograma, descripciones, alineaciones, esperanza(es decir, el umbral de significancia estadístico para reportar correspondencias versus secuencias de la base de datos), corte, matriz y filtro se encuentran en las configuraciones por omisión. La matriz de puntuación por omisión utilizada por blastp, blastx, tblastn y tblastx es la matriz BLOSUM62 (Henikoff, Proc. Natl. Acad. Sci. USA 89: 10915-10919 (1992), incorporada completamente como referencia). Para blastn, la matriz de puntuación es ajustada por la proporciones de M (es decir, el puntaje de retribución para un par de residuos correspondientes) a N (es decir, el puntaje por sanción para residuos no correspondientes), en donde los valores por omisión para M y N son 5 y 4, respectivamente. Cuatro parámetros blastn se pueden ajustar como sigue: Q=10 (sanción por creación de brechas); R=10 (sanción por extensión de brecha); wink=1 (genera acertadas de palabras en cada posición wink a lo largo de la consulta); y gapw= 16 (configura el ancho de ventana dentro de la cual se generan las alineaciones de brecha). Las configuraciones de parámetros Blastp equivalentes fueron Q=9; R=2; wink=1; y gapw=32. Una comparación Bestfit entre secuencias, disponible en la versión 10.0 del GCG package, utiliza los parámetros ADN GAP = 50 (sanción por creación de brecha) y LEN=3 (sanción por extensión de brecha) y las configuraciones equivalentes en comparaciones proteicas son GAP=8 y LEN=2.

Las variantes polinucleotidas del invento pueden contener alteraciones en regiones codificadoras, regiones no codificadoras, o ambas. Especialmente preferidas son las variantes polinucleotidas que contienen alteraciones que producen sustituciones silenciosas, adiciones, o eliminaciones, pero no alteran las propiedades o actividades del polipéptido codificado. Se prefieren las variantes nucleótidas producidas por sustituciones silenciosas debido a la degeneración del código genético. Además, también se prefieren las variantes polipeptídicas en las cuales menos de 50, menos de 40, menos de 30, menos de 20, menos de 10, o 5-50, 5-25, 5-10, 1-5, o 1-2 aminoácidos son sustituidos, eliminados, o agregados en cualquier combinación. Las variantes polinucleotidas se pueden producir por una variedad de razones, ej., para optimizar la expresión de codones para un huésped particular (cambiar codones en el mARN humano a aquellos preferidos por un huésped bacteriano, tal como, levadura o *E. coli).*

En una realización preferida, un Polinucleótido del invento el cual codifica la porción de albúmina de una proteína de fusión de albúmina es optimizada para expresión en células de levadura o mamíferas. En una realización preferida adicional, un polinucleótido del invento el cual codifica la porción de Proteína Terapéutica de una proteína de fusión de albúmina es optimizado para expresión en células de levadura o mamíferas. En aún una realización adicional, un Polinucleótido que codifica una proteína de fusión de albúmina del invento es optimizado para expresión en células de levadura o mamíferas.

En una realización alternativa, un Polinucleótido codón-optimizado el cual codifica una porción de proteína Terapéutica de una proteína de fusión de albúmina no hibridiza al Polinucleótido tipo silvestre que codifica la proteína Terapéutica bajo condiciones de hibridización severas como se describe en la presente. En una realización adicional, un Polinucleótido optimizado de codón el cual codifica una porción de albúmina de una proteína de fusión de albúmina no hibridiza al Polinucleótido tipo silvestre que codifica la proteína de albúmina bajo condiciones de hibridización severas tal como se describe en la presente.

En una realización adicional, un Polinucleótido que codifica una porción de proteína Terapéutica de una proteína de fusión de albúmina no comprende o alternativamente consta de la secuencia de ocurrencia natural de esa proteína Terapéutica. En una realización adicional, un Polinucleótido que codifica porción de proteína de albúmina de una proteína de fusión de albúmina no comprende o alternativamente consta de la secuencia de proteína de albúmina de ocurrencia natural. En una realización alternativa, un Polinucleótido que codifica una proteína de fusión de albúmina no comprende o alternativamente consta de la secuencia de ocurrencia natural de una porción de Proteína Terapéutica o la porción de proteína de albúmina.

Las variantes de ocurrencia natural son llamadas "variantes alélicas" y se refieren a una de las diferentes formas alternas de un gen que ocupa un sitio dado sobre un cromosoma de un organismo. (Genes II, Lewis, B. ed., John Wiley & Sons, Nueva Cork (1985)). Estas variantes alélicas pueden variar en el nivel polinucleotido y/o polipéptido y están incluidas en el presente invento. Alternativamente, las variantes de ocurrencia no natural se pueden producir mediante técnicas de mutagénesis o mediante síntesis directa.

Utilizando métodos conocidos de ingeniería de proteínas y tecnología de ADN recombinante, se pueden generar variantes para mejorar o alterar las características de los polipéptidos del presente invento. Por ejemplo, uno o más aminoácidos se pueden suprimir desde el termino N o termino C del polipéptido del presente invento sin perdida sustancial de función biológica. Como un ejemplo, Ron (J. Biol. Chem. 268: 2984-2988 (1993) reportó proteínas de la variante KGF con actividad de unión de heparina aún después de suprimir 3, 8, o 27 residuos de aminoácidos amino-terminales. De manera similar, la interferón gamma exhibió hasta diez veces

mayor actividad después de suprimir 8-10 residuos aminoácidos desde el término carboxilo de esta proteína. (Dobeli, J. Biotechnology 7:199-216 (1988)).

Además, amplia evidencia demuestra que las variantes a menudo retienen actividad biológica similar a aquella de la proteína de ocurrencia natural. Por ejemplo, Gayle y colegas 8J. Biol. Chem. 268:22105-22111 (1993)) realizaron extenso análisis de mutación de la citoquina humana IL-1a. Ellos utilizaron mutagénesis aleatoria para generar más de 3500 mutantes individuales IL-1a que promediaron 2.5 cambios de aminoácidos por variante sobre la longitud completa de la molécula. Se examinaron múltiples mutaciones en cada posición aminoácida posible. Los investigadores encontraron que "la mayoría de la molécula podría ser alterada con poco efecto sobre [unión o actividad biológica]. En realidad, solamente 23 secuencias de aminoácidos, entre más de 3500 secuencias nucleótidas examinadas, produjeron una proteína que fue significativamente diferente en actividad con respecto al tipo silvestre.

Además, aún si la eliminación de uno o más aminoácidos del término-N o término-C de un polipéptido resulta en modificación o pérdida de una o más funciones biológicas, otras actividades biológicas pueden aún ser retenidas. Por ejemplo, la capacidad de una variante de eliminación para inducir y/o unir anticuerpos los cuales reconocen la forma secretada probablemente será retenida cuando menos de la mayoría de los residuos de la forma secretada son removidos del término-N o término-C. Mediante métodos comunes descritos en la presente y de otra forma conocidos en el estado de la técnica se puede determinar si un polipéptido particular que carece de residuos N- o C-terminales de una proteína retiene tales actividades inmunogénicas.

Por lo tanto, el invento además incluye variantes polipeptidas que tienen una actividad funcional (ej., actividad biológica y/o actividad terapéutica). En una realización, el invento proporciona variantes de albúmina de proteínas de fusión de albúmina que tienen una actividad funcional (ej., actividad biológica y/o actividad terapéutica) que corresponde a una o más actividades biológicas y/o terapéuticas de la proteína terapéutica que corresponde a la porción de proteína Terapéutica de la proteína de fusión de albúmina. En otra realización, el invento proporciona variantes de proteínas de fusión de albúmina que tienen una actividad funcional (ej., actividad biológica y/o actividad terapéutica) que corresponde a una o más actividades biológicas y/o terapéuticas de la proteína Terapéutica correspondiente a la porción de proteína Terapéutica de la proteína de fusión de albúmina. Tales variantes incluyen eliminaciones, inserciones, inversiones, repeticiones, y sustituciones seleccionadas según las reglas generales conocidas en el estado de la técnica por tener poco efecto sobre actividad. Los polinucleótidos que codifican tales variantes también son contemplados por el invento.

En realizaciones preferidas, las variantes del invento tienen sustituciones conservadoras. "Sustituciones conservadoras" significan trueques dentro de grupos tal como reemplazo de los aminoácidos alifáticos o hidrofóbicos Ala, Val, Leu y Ile; reemplazo de residuos de hidroxilo Ser y Thr; reemplazo de residuos ácidos Asp y Glu; reemplazos de residuos de amida Asn y Gln, reemplazo de los residuos básicos Lys, Arg y His; reemplazo de los residuos aromáticos Phe, y Trp, y reemplazo de aminoácidos de tamaño pequeño Ala, Ser, Thr, Met, y Gly.

Se proporciona asesoramiento en cuanto a como preparar sustituciones silenciosas de aminoácidos, por ejemplo, en Bowie, "Descifrando el Mensaje en Secuencias de Proteínas: Tolerancia a Sustituciones de Aminoácidos," Science 247:1306-1310 (1990), en donde los autores indican que existen dos estrategias principales para estudiar la tolerancia de una secuencia de aminoácidos a cambiar.

La primera estrategia explota la tolerancia de sustituciones de aminoácidos mediante selección natural durante el proceso de evolución. Al comparar las secuencias aminoácidas en

diferentes especies, se pueden identificar aminoácidos conservados. Estos aminoácidos conservados probablemente son importantes para función de proteínas. En contraste, las posiciones aminoácidas donde las sustituciones han sido toleradas mediante selección natural indican que estas posiciones no son críticas para función de proteínas. Por lo tanto, las posiciones que toleran sustitución de aminoácidos pueden ser modificadas mientras se sigue manteniendo actividad biológica de la proteína.

Como afirman los autores, estas dos estrategias han revelado que las proteínas son sorprendentemente tolerantes a sustituciones de aminoácidos. Los autores además indican cuales cambios aminoácidos probablemente van a ser permisivos en ciertas posiciones aminoácidas en la proteína. Por ejemplo, los residuos aminoácidos más profundos (dentro de la estructura terciaria de la proteína) requieren cadenas laterales no polares, mientras pocas características de cadenas laterales de superficie generalmente son conservadas. Además, las sustituciones aminoácidas conservadoras toleradas involucran reemplazo de aminoácidos alifáticos o hidrofóbicos Ala, Val, Leu y Ile; reemplazo de residuos de hidróxilo Ser y Thr; reemplazo de residuos ácidos Asp y Glu; reemplazo de los residuos de amida Asn y Gln, reemplazo de los residuos básicos Lys, Arg, y His; reemplazo de los residuos aromáticos Phe, Tyr, y Trp, y reemplazo de los aminoácidos de tamaño pequeño Ala, Ser, Thr, Met, y Gly. Además de la sustitución aminoácida conservadora, las variantes del presente invento incluyen (i) polipéptidos que contienen sustituciones de uno o más de los residuos aminoácidos no conservados, donde los residuos aminoácidos sustituidos pueden o no ser codificados mediante el código genético, o (ii) polipéptidos que contienen sustituciones de uno o más de los residuos aminoácidos con un grupo sustituyente, o (iii) polipéptidos que han sido fusionados con o químicamente conjugados a otro compuesto, tal como un compuesto para aumentar la estabilidad y/o solubilidad del polipéptido (por ejemplo, glicol de polietileno), (iv) polipéptidos que contienen aminoácidos adicionales, tal como por ejemplo, polipéptido de región de fusión IgG Fc. Tales polipéptidos variantes se consideran dentro del alcance de aquellos con experiencia en el estado de la técnica a partir de las enseñanzas en la presente.

Por ejemplo, las variantes polipeptídicas que contienen sustituciones aminoácidas de aminoácidos cargados con otros aminoácidos cargados o neutrales pueden producir proteínas con características mejoradas. Tal como menos agregación. La agregación de fórmulaciones farmacéuticas reduce actividad y aumenta eliminación debido a la actividad inmunogénica del agregado. Ver Pinckard, Clin. Exp. Immunol. 2:331-340 (1967); Robbins, Diabetes 36: 838-845 (1987), Cleland, Crit. Rev. Sistemas de Vehículos Farmacéuticos Terapéuticos 10:307-377 (1993).

En realizaciones específicas, los polipéptidos de invento comprenden, o alternativamente constan de fragmentos o variantes de la secuencia de aminoácidos de una proteína de fusión de albúmina, la secuencia de aminoácidos de una proteína terapéutica y/o albúmina sérica humana, en donde los fragmentos o variantes tienen 1-5, 5-10, 5-25, 5-50, 10-50 o 50-150 adiciones, sustituciones, y/o eliminaciones de residuos aminoácidos en comparación con la secuencia de aminoácidos de referencia. En realizaciones preferidas, las sustituciones aminoácidas son conservadoras. Los ácidos nucleicos que codifican éstos polipéptidos también son contemplados por el invento.

El polipéptido del presente invento puede estar compuesto de aminoácidos unidos entre sí mediante enlaces péptidos o enlaces péptidos modificados, es decir, péptidos isósteros, y puede contener aminoácidos diferentes a los 20 aminoácidos codificados por el genoma. Los polipéptidos se pueden modificar ya sea mediante procesos naturales, tal como procesamiento post-traslacional, o mediante técnicas de modificación química las cuales son bien conocidas en

el estado de la técnica. Tales modificaciones se describen en textos básicos y en monografías más detalladas, así como también en literatura de investigación voluminosa. Las modificaciones pueden ocurrir en cualquier parte en un polipéptido, incluyendo el esqueleto del péptido, las cadenas laterales aminoácidas y los términos amino o carboxilo. Se apreciará que el mismo tipo de modificación puede estar presente en grados iguales o variables en varios sitios en un polipéptido dado. También, un polipéptido dado puede contener muchos tipos de modificaciones. Los polipéptidos pueden ser ramificados, por ejemplo, como resultado de ubiquitinación, y pueden ser cíclicos, con o sin ramificación. Los polipéptidos cíclicos, ramificados, y cíclicos ramificados pueden resultar a partir de procesos naturales de postraducción o se pueden preparar mediante métodos sintéticos. Las modificaciones incluyen acetilación, acilación, ADP-ribosilación, amidación, fijación covalente de flavina, fijación covalente de una fracción heme, fijación covalente de un nucleótido o derivado nucleótido, fijación covalente de un lípido o derivado lípido, fijación covalente de fosfotidilinositol, entrecruzamiento, ciclización, formación de enlaces de disulfuro, desmetilación, formación de eslabones covalentes, formación cisteína, formación de piroglutamato, formilación, gama-carboxilación, glicosilación, formación de anclas GPI, hidroxilación, yodinación, metilación, miristilación, oxidación, pegilación, procesamiento proteolítico, fosforilación, prenilación, racemización, selenoilación, sulfatación, adición de aminoácidos a proteínas mediada por transferecia de ARN tal como arginilación, y ubiquitinación. (Ver, por ejemplo, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POST-TRANSLATIONAL COVALENT MODFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs 1-12 (1983); Seifer, Meth. Enzymol. 182:626-646 (1990); Rattan, Ann. N.Y. Acad. Sci. 663:48-62 (1992)).

Actividad funcional

"Un polipéptido con actividad funcional" se refiere a un polipéptido capaz de presentar una o más actividades funcionales conocidas asociadas con la pro-proteína de longitud completa, y/o forma madura de una proteína terapéutica. Tales actividades funcionales incluyen, pero no están limitadas a actividad biológica, antigenicidad [capacidad para unirse (o competir con un polipéptido por unión) a un anticuerpo anti-péptido], inmunogenicidad (capacidad para generar un anticuerpo el cual se une a un polipéptido específico del invento), capacidad para formar multímeros con polipéptidos del invento, y capacidad para unirse a un receptor o ligando para un polipéptido.

"Un polipéptido con actividad biológica" se refiere a un polipéptido que exhibe actividad similar, pero no necesariamente idéntica a una actividad de una proteína terapéutica del presente invento, incluyendo formas maduras, tal como se midió en un ensayo biológico particular, con o sin dependencia de dosis. En el caso donde existe dependencia de dosis, no necesita ser idéntica a aquella del polipéptido, pero en cambio sustancialmente similar a la dependencia de dosis en una actividad dada en comparación con el polipéptido del presente invento (es decir, el polipéptido candidato, exhibirá mayor actividad o no más de 25 veces menos aproximadamente y, preferiblemente, no más de diez veces menos actividad aproximadamente, y más preferiblemente, no más de tres veces menos actividad aproximadamente con relación al polipéptido del presente invento.

En realizaciones preferidas, una proteína de fusión de albúmina del invento tiene al menos una actividad biológica y/o terapéutica asociada con la porción de proteína terapéutica (o fragmento o variante de la misma) cuando no está fusionada a albúmina.

En realizaciones preferidas adicionales, la proteína de fusión de albúmina del invento tiene estabilidad del plasma aumentada en comparación con la porción de proteína terapéutica (o

fragmento o variante de la misma) en un estado sin funcionar. La estabilidad del plasma de la proteína de fusión de albúmina del invento o de la porción de proteína terapéutica sin funcionar (o fragmento o variante de la misma) pueden evaluarse utilizando o modificando de manera rutinaria los ensayos conocidos en el estado de la técnica.

Las proteínas de fusión de albúmina del invento pueden ser probadas en cuanto a actividad funcional (ej., actividad biológica) utilizando ensayos rutinariamente modificadores conocidos en el estado de la técnica, así como también ensayos descritos en la presente. Adicionalmente, alguien con experiencia en el estado de la técnica puede probar rutinariamente fragmentos de una proteína terapéutica correspondiente a una porción de proteína terapéutica de una proteína de fusión de albúmina, para actividad utilizando ensayos citados en su fila correspondiente en la tabla 1 (ej., en columna 3 de Tabla 1). Además, alguien con experiencia en el estado técnica puede probar rutinariamente fragmentos de una proteína de albúmina correspondiente a una porción de proteína de albúmina de una proteína de fusión de albúmina para actividad utilizando ensayos conocidos en el estado técnica y/o tal como se describe en la sección de ejemplos más adelante.

Por ejemplo, en una realización donde se prueba la capacidad de una proteína de fusión de albúmina para unirse o competir con la proteína terapéutica para unión a un anticuerpo polipéptido anti-terapéutico y/o anticuerpo anti-albúmina, se puede utilizar varios inmunoensayos conocidos en el estado de la técnica, incluyendo pero sin limitar a sistemas de ensayos competitivos y no competitivos utilizando técnicas tales como radioinmunoensayos, ELISA (ensayo inmunosorbente ligado a enzima), inmunoensayos tipo "sandwich", ensayos inmunoradiométricos, reacciones de precipitación mediante difusión de gel, ensayos de inmunodifusión, inmunoensayos in situ (utilizando oro coloidal, rótulos enzimáticos o radioisotópicos, por ejemplo), western blots, reacciones de precipitación, ensayos de aglutinación, ensayos de hemaaglutinación, ensayos de fijación de complemento, ensayos de inmunofluorescencia, ensayos de proteína A, ensayos de inmunoelectroforesis, etc. en una realización, la unión de anticuerpos es detectada al detectar un rótulo sobre el anticuerpo primario. En otra realización, el anticuerpo primario es detectado al detectar unión de un anticuerpo secundario o reactivo al anticuerpo primario. En una realización adicional, el anticuerpo secundario es rotulado. Muchos medios son conocidos en el estado de la técnica para detectar unión en un inmunoensayo y están dentro del alcance del presente invento.

En una realización preferida, donde se identifica un socio de fijación, (ej., un receptor o un ligando) de una proteína terapéutica, se puede probar la fijación a ese socio de fijación mediante una proteína de fusión de albúmina que comprende esa proteína terapéutica como la porción de proteína terapéutica de la fusión, ej., por medios conocidos en el estado de la técnica, tal como, por ejemplo, cromatografía de gel reductora y no reductora, cromatografía de afinidad proteica, y blotting por afinidad. Ver generalmente, Phizicky, Microbiol. Rev. 59:94-123 (1995). En otra realización, la capacidad de correlatos fisiológicos de una proteína de fusión de albúmina para unirse a un sustrato del polipéptido terapéutico correspondiente a la porción de proteína terapéutica de la fusión se puede probar de manera rutinaria utilizando técnicas conocidas en el estado de la técnica.

En una realización alternativa, donde la capacidad de una proteína de fusión de albúmina para multimerizar es evaluada, la asociación con otros componentes del multímero se puede probar ej. por medios bien conocidos en el estado de la técnica, tal como por ejemplo cromatografía de gel reductora y no reductora, cromatografía de afinidad de proteínas, y secado de afinidad. Ver generalmente, Phizicky, anteriormente.

En realizaciones preferidas una proteína de fusión de albúmina que comprende todo o una porción de un anticuerpo que fija una proteína terapéutica, tiene al menos una actividad biológica y/o terapéutica (ej., para unir específicamente un polipéptido o epítope) asociada con el anticuerpo que fija una proteína terapéutica (o fragmento o variante de la misma) cuando no está fusionada a albúmina. En otros realizaciones preferidas, la actividad biológica y/o actividad terapéutica de una proteína de fusión de albúmina que comprende todo o una parte de un anticuerpo que fija una proteína terapéutica es la inhibición (es decir, antagonismo) o activación (es decir, agonismo) de una o más actividades biológicas y/o actividades terapéuticas asociadas con un polipéptido que es específicamente unido por el anticuerpo que fija una proteína terapéutica.

Las proteínas de fusión de albúmina comprendiendo al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica se pueden caracterizar en una variedad de formas. En particular, las proteínas de fusión de albúmina comprendiendo al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica pueden ser probadas en cuanto a la capacidad para unirse específicamente a los mismos antígenos específicamente unidos por el anticuerpo que fija una proteína terapéutica correspondiente a la porción de proteína terapéutica de la proteína de fusión de albúmina utilizando técnicas descritas en la presente o mediante técnicas rutinariamente modificadoras conocidas en el estado de la técnica.

Ensayos para capacidad de las proteinas de fusión de albúmina (ej., comprendiendo al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica) para unir (específicamente) una proteína específica o epítope, se pueden realizar en solución (ej., Houghten, Bio/Tecniques 13:412-42(1992)), sobre perlas (*ej.* Lam, Nature 354:82-84 (1991)), en hojuelas (ej., Fodor, Nature 364:555-556 (1993)), sobre bacterias (ej., Patente Americana No. 5.223.409), sobre esporas (ej., Patentes No. 5.571.698; 5.403.484; y 5.223.409), sobre plásmidos (ej., Cull., Proc. Natl. Acad. Sci. USA 89:1865-1869 (1992) o sobre fagos (ej., Scott y Smith, Science 249:386-390 (1990); Devlin, Science 249:404-406 (1990); Cwirla, Proc. Natl. Acad. Sci. USA 87:6378-6382 (1990); y Felici, J. Mol. Biol. 222:301-310 (1991)) (cada una de estas referencias es incorporada en la presente como referencia en su totalidad). Las proteínas de fusión de albúmina comprendiendo al menos un fragmento o variante de un anticuerpo terapéutico también se pueden probar en cuanto a su especificidad y afinidad para una proteína específica o epítope utilizando técnicas rutinariamente modificadoras descritas en la presente o de otra manera conocidas en el estado de la técnica.

Las proteínas de fusión de albúmina comprendiendo al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica se pueden probar en cuanto a reactividad cruzada con otros antígenos (ej., moléculas que tienen conservación de secuencia/estructura con la molécula específicamente unida por el anticuerpo que fija una proteína terapéutica (o fragmento o variante de la misma) correspondiente a la porción de proteína terapéutica de la proteína de fusión de albúmina de invento) mediante cualquier método conocido en el estado de la técnica.

Los inmunoensayos que se pueden tuilizar para analizar fijación y reactividad cruzada incluyen, pero no están limitados a sistemas de ensayos competitivos y no competitivos que utilizan técnicas tales como western blots, radioinmunoensayos, ELISA (ensayo inmunosorbente ligado a enzima), inmunoensayos tipo "sandwich", ensayos de inmunoprecipitación, reacciones de precipitación, reacciones de precipitación mediante difusión de gel, ensayos de inmunodifusión, ensayos de aglutinación, ensayos de fijación de complemento, ensayos inmunoradiométricos, inmunoensayos florescentes, e inmunoensayos de proteína A, por nombrar solo algunos. Tales ensayos son comunes y son bien conocidos en el estado de la técnica (ver, *ej.*, Ausubel, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc.,

New York, el cual se incorpora como referencia en la presente en su totalidad). Inmunoensayos de ejemplo se describen brevemente a continuación (pero no pretenden ser limitantes).

Los protocolos de inmunoprecipitación generalmente comprenden lisis de una población de células en un amortiguador de lisis tal como amortiguador RIPA (1% NP-40 o Triton X-100, 1% deoxicolato de sodio, 0.1% SDS, 0.15 M NaCl, 0.01 M fosfato de sodio a pH 7.2, 1% Trasylol) suplementado con inhibidores de la proteína-fosfatasa y/o proteasa (ej., EDTA, PMSF, aprotinina, vanadato de sodio), agregando la proteina de fusión de albumina del invento (ej., comprendiendo al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica) al lisado celular, incubando durante un periodo de tiempo (ej., 1 a 4 horas) a 40 grados centigrados, agregando perlas de sefarosa acopladas a un anticuerpo anti-albúmina, por ejemplo, al lisado celular, incubando durante aproximadamente una hora o más a 40 grados C, lavando las perlas en amortiguador de lisis y resuspendiendo las perlas en el amortiguador de SDS/muestra. La capacidad de la proteína de fusión de albúmina para inmunoprecipitar un antígeno particular se puede evaluar mediante ej., análisis por western blotting. Alguien con experiencia en el estado de la técnica conocerá cuales parámetros pueden ser modificados para aumentar la unión de la proteína de fusión de albúmina a un antígeno y disminuir el entorno (ej., pre-clarificación de lisado celular con perlas de sefarosa). Para discusión adicional con respecto a protocolos de inmunoprecipitación ver, ej., Ausubel eds, 1994, Current Protocols in Molecular Biology, Vol 1. John Wiley & Sons, Inc., New York 10.16.1.

El análisis western blot generalmente comprende preparación de muestras proteicas, electroforesis de las muestras proteicas en un gel de poliacrilamida (ej., 8%-20% SDS-PAGE dependiendo del peso molecular del antígeno), transferencia de la muestra de proteínas desde el gel de poliacrilamida a una membrana tal como nitrocelulosa, PVDF o nailon, bloqueo de la membrana en solución bloqueadora (ej., PBS con BSA al 3% o leche no grasa), lavado de la membrana en amortiguador de lavado (ej., PBS-Tween 20), aplicación de la proteína de fusión de albúmina del invento (diluida en amortiguador bloqueador) a la membrana, lavado de la membrana en amortiguador de lavado, aplicación de un anticuerpo secundario (el cual reconoce a proteína de fusión de albúmina, ej., un anticuerpo de albúmina sérica anti-humano) conjugado a un sustrato enzimático (ej., peroxidasa de rábano o fosfatasa alcalina) o molecula radioactiva (ej., 32P o 125I) diluida en amortiguador bloqueador, lavando la membrana en amortiguador de lavado, y detectando la presencia del antígeno. Alguien con experiencia en el estado de la técnica conocerá los parámetros que se pueden modificar para aumentar la señal detectada y reducir el ruido de entorno. Para discusión adicional con respecto a los protocolos western blot, ver *ej.*, Ausubel, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York en 10.8.1.

ELISAs comprenden la preparación de antigenos, recubrimiento del pozo de una placa de microtitulos de 96 pozos con el antígeno, lavado del antigeno que no se unió a los pozos, adición de proteína fusión de albúmina (ej., comprendiendo al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica) del invento conjugada a un compuesto detectable tal como un sustrato enzimático (ej., peroxidasa de rábano o fosfatasa alcalina) a los pozos e incubación durante un período de tiempo, lavando las proteínas de fusión de albúmina no unidas o no específicamente unidas, y detectando la presencia de las proteínas de fusión de albúmina específicamente unidas al antígeno recubriendo el pozo. En ELISAs la proteína de fusión de albúmina no tiene que ser conjugada a un compuesto detectable; en cambio, un segundo anticuerpo (el cual reconoce la proteína de fusión de albúmina) conjugado a un compuesto detectable puede ser agregado al pozo. Además, en cambio de recubrir pozo con el antígeno, la proteína de fusión de albúmina puede ser impregnada al pozo. En este caso, la molécula

detectable puede ser el antígeno conjugado a un compuesto detectable tal como un sustrato enzimático (*ej.*, peroxidasa de rábano o fosfatasa alcalina). Alguien con experiencia en el estado de la técnica conocerá cuales parámetros pueden ser modificados para aumentar la señal detectada así como también otras variaciones de ELISAs conocidas en el estado de técnica. Para discusión adicional con respecto a ELISAs ver, Ausubel, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York en 11.2.1.

La afinidad de fijación de una proteína de fusión de albúmina a una proteína, antígeno, o epítope y la tasa de disociación de una interacción de proteína de fusión de albúmina-proteína/antígeno/epítope se puede determinar mediante ensayos de unión competitivos. Un ejemplo de un ensayo de unión competitivo es un radioinmunoensayo que comprende la incubación del antígeno rotulado (ej., 3H o 125I) con la proteína de fusión de albúmina del invento en presencia de cantidades crecientes de antígeno no rotulado, y la detección del anticuerpo unido al antígeno rotulado. La afinidad de la proteína de fusión de albúmina para una proteína específica, antígeno, o epítope y las tasas-off de unión se puede determinar a partir de los datos mediante análisis Scatchard plot. La competición con una segunda proteína que fija la misma proteína, antígeno o epítope que la proteína de fusión de albúmina, también se puede determinar utilizando radioinmunoensayos. En este caso, la proteína, antígeno o epítope se incubada con una proteína de fusión de albúmina conjugada a un compuesto rotulado (ej., 3H o 125I) en la presencia de cantidades crecientes de una segunda proteína sin rotular que fija la misma proteína, antígeno o epítope que la proteína de fusión de albúmina del invento.

En la realización preferida, el análisis cinético BIAcore se utiliza para determinar la fijación y las tasas de disociación de proteínas de fusión de albúmina del invento a una proteína, antígeno o epítope. El análisis cinético BIAcore comprende análisis de fijación y disociación de proteínas de fusión de albúmina, o polipéptidos específicos, antígenos o epítopes a partir de hojuelas con polipéptidos específicos inmovilizados, antígenos o epítopes o proteínas de fusión de albúmina, respectivamente, sobre su superficie.

Los anticuerpos que fijan una proteína terapéutica correspondiente a la porción de proteína terapéutica de una proteína de fusión de albúmina también se pueden describir o especificar en términos de su afinidad de unión para un antígeno o proteína dada, preferiblemente el antígeno que específicamente unen. Afinidades de unión preferidas incluyen aquellas con una constante de disociación o Kd menor que 5 x 10^{-2}M, 10^{-2}M, 5x10^{-3}M, 10^{-3}M, 5X10^{-4}M, 10^{-4}M. Afinidades de unión más preferidas incluyen aquellas con una constante de disociación Kd menor que 5 X10^{-5}M, 10^{-5}M, 5 X 10^{-6}M, 10^{-6}M, 5X10^{-7}M, 10^{7}M, 5M, 10^{-10}M, 5 X10^{-11}M, 10^{-11}M, 5 X 10^{-12}M, 10^{-12}M, 5X10^{-13}M, 10^{-13}M, 5 x10-^{14}M, 10^{-14}M, 5X10^{-15}M, o 10^{-15}M. En realizaciones preferidas, las proteínas de fusión de albúmina que comprenden al menos un fragmento o variante del anticuerpo que fija una proteína terapéutica, tienen una afinidad para una proteína o epítope dado similar a aquella del anticuerpo correspondiente (no fusionado a albúmina) que fija la proteína terapéutica, tomando en cuenta la valencia en la proteína de fusión de albúmina (comprendiendo al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica) y la valencia del anticuerpo correspondiente. Además, los ensayos descritos en la presente (ver ejemplos y tabla 1) y de otra manera conocidos en el estado de la técnica se pueden aplicar de manera común para medir la capacidad de las proteínas de fusión de albúmina y fragmentos, variantes y derivados de las mismas para producir actividad biológica y/o actividad terapéutica (ya sea *in vivo o in vitro*) relacionada con la porción de proteína terapéutica y/o porción de albúmina de la proteína de fusión de albúmina. Otros métodos serán conocidos por aquellos con experiencia y están dentro del alcance del invento.

Albumina

Como se describió anteriormente, una proteína de fusión de albúmina del invento comprende al menos un fragmento o variante de una proteína terapéutica y al menos un fragmento o variante de albúmina sérica humana, las cuales son asociadas entre sí, preferiblemente mediante fusión genética.

Una realización adicional comprende al menos un fragmento o variante de una proteína terapéutica y al menos un fragmento o variante de albúmina sérica humana, las cuales están unidas entre sí mediante conjugación química.

Los términos, albúmina sérica humana (HSA) y albúmina humana (HA) son utilizados de manera intercambiable en la presente. Los términos, "albúmina y albúmina sérica" son más amplios, e incluyen albúmina sérica humana (y fragmentos y variantes de la misma) así como también albúmina de otras especies (y fragmentos y variantes de la misma).

Como se utiliza la presente, "albúmina" se refiere colectivamente a proteína de albúmina o secuencia de aminoácidos, o fragmento o variante de albúmina, con una o más actividades funcionales (ej., actividades biológicas) de albúmina. En particular, "albúmina" se refiere a albúmina humana o fragmentos de la misma (ver por ejemplo, EP 201 239, EP 322 094 WO 97/24445, WO95/23857) especialmente la forma madura de albúmina humana como se muestra en la figura 1 y SEQ ID NO:1, o albúmina de otros vertebrados o fragmentos de la misma, o análogos o variantes de estas moléculas o fragmentos de las mismas.

En realizaciones preferidas, la proteína de albúmina sérica humana utilizada en las proteínas de fusión de albúmina del invento contiene uno o ambos de los siguientes grupos de mutaciones de punto con referencia a SEQ ID NO: 1: Leu-407 a Ala, Leu-408 a Val, Val.409 a Ala, y Arg-410 a Ala; o Arg-410 a A, Lys-413 a Gln, y Lys-414 a Gln (ver, ej., Publicación Internacional No. WO95/23857, incorporada en la presente como referencia en su totalidad). En realización aún más preferidas, las proteínas de fusión de albúmina del invento que contienen uno o ambos grupos anteriormente descritos de mutaciones de punto tienen resistencia/estabilidad mejoradas a eliminación proteolítica de levadura Yap3p, permitiendo producción aumentada de proteínas de fusión de albúmina recombinantes expresadas en células huésped de levadura.

Como se utiliza en la presente, una porción de albúmina suficiente para prolongar la actividad terapéutica o estabilidad plasmática o vida en almacenamiento de la proteína terapéutica se refiere a una porción de albúmina suficiente en longitud o estructura para estabilizar o prolongar la actividad terapéutica o estabilidad plasmática de la proteína de manera que la vida en almacenamiento o estabilidad plasmática de la porción de proteína terapéutica de la proteína de fusión de albúmina es prolongada o extendida en comparación con la vida en almacenamiento o estabilidad plasmática en el estado de no fusión. La porción de albúmina de las proteínas de fusión de albúmina puede comprender la longitud completa de la secuencia HA como se describió anteriormente, o puede incluir uno o más fragmentos de la misma que son capaces de estabilizar o prolongar la actividad terapéutica. Tales fragmentos pueden ser de 10 o más aminoácidos de longitud o pueden incluir aproximadamente 15, 20, 25, 30, 50, o más aminoácidos contiguos desde la secuencia HA o pueden incluir parte o todos los dominios específicos de HA. Por ejemplo, se pueden utilizar uno o más fragmentos de HA que abarcan los dos primeros dominios tipo inmunoglobulina. En una realización preferida, el fragmento HA es la forma madura de HA.

La porción de albúmina de las proteínas de fusión de albúmina del invento puede ser una variante de HA normal. La porción de proteína terapéutica de las proteínas de fusión de albúmina del invento también pueden ser variantes de las proteínas terapéuticas tal como se describe en la presente. El término "variantes" incluye inserciones, eliminaciones y sustituciones,

ya sea conservadoras o no conservadoras, donde tales cambios no alteran sustancialmente una o más de las propiedades oncóticas, de unión de ligandos útiles y no inmunogénicas de albúmina, o el sitio activo, o dominio activo el cual confiere las actividades terapéuticas de las proteínas terapéuticas.

En particular, las proteínas de fusión de albúmina del invento pueden incluir variantes polimórficas de ocurrencia natural de albúmina humana y fragmentos de albúmina humana, por ejemplo aquellos fragmentos divulgados en EP 322 094 (a saber HA (Pn), donde n es 369 a 419). La albúmina se puede derivar a partir cualquier vertebrado, especialmente cualquier mamífero, por ejemplo humano, vaca, oveja, o cerdo. Albúminas no mamíferas incluyen, pero no están limitadas a gallina y salmón. La porción de albúmina de la proteína de fusión de albúmina puede provenir de cualquier animal diferente que la porción de proteína terapéutica.

Generalmente, un fragmento o variante de HA tendrá al menos 100 aminoácidos de longitud, preferiblemente al menos 150 aminoácidos. La variante HA puede constar o alternativamente comprender al menos un dominio entero de HA, por ejemplo dominio 1 (aminoácidos 1-194 de SEQ ID NO:1), dominio 2 (aminoácidos 195-387 de SEQ IDS NO:1), dominio 3 (aminoácidos 388-585 ID NO:1), dominios 1 y 2 (1-387 de SEQ ID NO:1), dominios 2 y 3 (195-585 de SEQ ID NO:1) o dominios 1 y 3 (aminoácidos 1-194 de SEQ ID NO: 1 y aminoácidos 388-585 de SEQ ID NO:1). Cada dominio se compone así mismo de dos subdominios homólogos a saber 1-105, 120-194, 195-291, 316-387, 388-491 y 512-585, con regiones enlazadoras flexibles entre subdominios comprendiendo los residuos Lys106 a Glu119, Glu292 a Val315 y Glu492 a Ala511.

Preferiblemente, la porción de albúmina de una proteína de fusión de albúmina del invento comprende al menos un subdominio o dominio de HA o modificaciones conservadoras de los mismos. Si la fusión se basa en subdominios, parte o todo el enlazador adyacente se utiliza preferiblemente para enlazar a la fracción de proteína terapéutica.

Los anticuerpos que específicamente unen proteínas terapéuticas también son proteínas terapéuticas

El presente invento también contempla proteínas de fusión de albúmina que comprenden al menos un fragmento o variante de un anticuerpo que específicamente une una proteína terapéutica divulgada tabla 1. Específicamente se contempla que el término "proteína terapéutica" incluye anticuerpos que fijan una proteína terapéutica (ej., como se describe en la columna 1 de la tabla 1) y fragmentos y variantes de los mismos. Así una proteína de fusión de albúmina del invento puede contener al menos un fragmento o variante de una proteína terapéutica, y/o al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica.

Estructura y fundamento de anticuerpos

Se sabe que la unidad estructural básica de anticuerpos comprende un tetrámero. Cada tetrámero está compuesto de dos pares idénticos de cadenas polipeptídicas, cada par con una cadena "liviana" (aproximadamente 25 kDa) y una cadena "pesada" (aproximadamente 50-70 KDa). La porción aminoterminal de cada cadena incluye una región variable de aproximadamente 100 a 110 o más aminoácidos principalmente responsables de reconocimiento de antígenos. La porción carboxi-terminal de cada cadena define una región constante principalmente responsable de la función efectora. Las cadenas livianas humanas se clasifican como cadenas livianas kappa y lambda. Las cadenas pesadas se clasifican como mu, delta, gama, alfa, o epsilón, y definen el isotipo del anticuerpo como IgM, IgD, IgG, IgA, y IgE, respectivamente. Ver generalmente, *Fundamental Immunology* Capitulos 3-5 (Paul, W., ed. 4th ed. Raven Press, N.Y. (1998)) (incorporado como referencia en su totalidad para todo propósito).

Las regiones variables de cada par de cadenas liviana/pesada forman el sitio de unión de anticuerpos.

Por lo tanto, el anticuerpo IgG intacto tiene dos sitios de unión. Excepto en anticuerpos bifuncionales o biespecíficos, los dos sitios de unión son los mismos.

Las cadenas todas exhiben la misma estructura general de regiones de marco relativamente conservadoras (FR) unidas por tres regiones hipervariables, también llamadas regiones determinadoras de complementariedad o CDRs. Las regiones CDR, en general, son las porciones del anticuerpo que hacen contacto con el antígeno y determinan su especificidad. Las CDRs de cadenas pesadas y livianas de cada par están alineadas por las regiones de marco, permitiendo unión a un epítope específico. Desde el término N hasta el término C, ambas regiones variables de cadenas liviana y pesada comprenden los dominios FR1, CDR1, FR2, CDR2, FR3, CDR3 y FR4. Las regiones variables están conectadas a la región constante de cadena pesada o liviana. La asignación de aminoácidos a cada dominio se hace según las definiciones de Kabat *Sequences of Proteins of Inmmunological Interes* (National Institutes of Health, Bethesda, Md. (1987 y 1991))), o Chothia & Lesk J *Mol. Biol.* 196:901-917 (1987); Chothia *Nature* 342:878-883 (1989).

Como se utiliza en la presente, "anticuerpo" se refiere a moléculas de inmunoglobulina y porciones inmunologicamente activas de moléculas de inmunoglobulina, es decir, moleculas que contienen un sitio de unión de antígenos que especificamente unen un antígeno (ej., una molecula que contiene una o más regiones CDR de un anticuerpo). Los anticuerpos que pueden corresponder a un porción de proteina Terapeutica de una proteina de fusión de albumina incluyen, pero no están limitados a anticuerpos monoclonales, multiespecíficos, humanos, humanizados o quiméricos, anticuerpos de cadena simple (ej., cadena simple Fvs), framentos fab, fragmentos f(ab'), fragmentos producidos por una biblioteca de expresión Fab, anticuerpos antiidiotípicos (anti-Id) (incluyendo, ej., anticuerpos anti-Id específicos a anticuerpos del invento), y fragmentos de unión de epítopes de cualquiera de los anteriores (ej., dominiones VH, dominios VL, o una o más regiones CDR).

Anticuerpos que fijan Proteínas Terapeuticas

El presente invento incluye proteínas de fusión de albúmina que comprenden al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica (ej., como se divulga en la Tabla 1) o fragmento o variante de la misma.

Los anticuerpos que fijan una proteína terapéutica (o fragmento o variante de la misma) pueden provenir de cualquier origen animal, incluyendo aves y mamíferos. Preferiblemente, los anticuerpos son humanos, murinos (ej., ratón y rata), anticuerpos de asno, oveja, conejo, cabra, conejillo de Indias, camello, caballo, o pollo. Más preferiblemente, los anticuerpos son anticuerpos humanos. Como se utiliza en la presente, anticuerpos "humanos" incluyen anticuerpos con la secuencia de aminoácidos de una inmunoglobulina humana e incluyen anticuerpos aislados desde bibliotecas de inmunoglobulina humana y xenoratones u otros orgánismos que han sido diseñados geneticamente para producir anticuerpos humanos.

Las moléculas de anticuerpos que fijan una proteína terapéutica y que pueden corresponder a una porción de proteína terapéutica de una proteína de fusión de albúmina del invento pueden ser de cualquier tipo (ej., IgG, IgE, IgM, IgD, IgA, e IgY), clase (ej., IgG1, IgG2, IgG3, IgG4, IgA1 y IgA2) o subclase de molecula de inmunoglobulina. En realizaciones preferidas, las moleculas de anticuerpo que se unen a una proteina terapeutica y que pueden corresponder a una porción de proteína terapéutica de una proteína fusión de albúmina son IgG1. En otras realizaciones preferidas, las moléculas de inmunoglobulina que se unen a una proteína

terapéutica y que pueden corresponder a una porción de proteína terapéutica de una proteína de fusión de albúmina son IgG4.

Más preferiblemente los anticuerpos que se unen a una proteína terapéutica y que pueden corresponder a una porción de proteína terapéutica de una proteína de fusión de albúmina son fragmentos de anticuerpos de unión de antígenos humanos del presente invento e incluyen, pero no están limitados a Fab, Fab', y F(ab')2, Fd, Fvs de cadena simple (scFv), anticuerpos de cadena simple, fvs unidos a disulfuro (sdFv) y fragmentos que comprenden un dominio VL o VH. Los fragmentos de anticuerpos de unión de antígenos, incluyendo anticuerpos de cadena simple, pueden comprender la región variable sola o en combinación con la totalidad o una porción de lo siguiente: región de bisagra, dominios CH1, CH2, y CH3.

Los anticuerpos que se unen a una proteína terapéutica y que pueden corresponder a una porción de proteína terapéutica de una proteína de fusión de albúmina pueden ser monoespecíficos, biespecíficos, triespecíficos o de mayor multiespecificidad. Los anticuerpos multiespecíficos pueden ser específicos para diferentes epítopes de una proteína terapéutica o pueden ser específicos tanto para una proteína terapéutica como para un epítope heterólogo, tal como un polipéptido heterólogo un material de soporte sólido. Ver, ej., Publicaciones PCT WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793, Tutt, J. Inmmunol. 147:60-69 (1991); Patente Americana No. 4.474.893; 4.714.681; 4.925.648; 5.573.920; 5.601.819; Kostelny, J. Immunol. 148:1547-1553 (1992).

Los anticuerpos que se unen a una proteína terapéutica (o fragmento o variante de la misma) pueden ser biespecíficos o bifuncionales lo cual significa que en anticuerpo es un anticuerpo híbrido artificial con dos pares de cadenas pesada/liviana diferentes y dos sitios de unión diferentes. Los anticuerpos biespecíficos se pueden producir mediante una variedad de métodos incluyendo fusión de hibridomas o entrecruzamiento de fragmentos Fab'. *Ver ej.*, Songsivilai & Lachmann *Clin. Exp. Immnol.* 79: 315-321 (1990), Kostelny J. Immunol. 148:1547 1553 (1992). Además, los anticuerpos biespecíficos se pueden formar como "diacuerpos" (Holliger ""Diacuerpos": fragmentos de anticuerpos bivalentes y biespecíficos pequeños" PNAS USA 90:6444-6448 (1993) o "Janusins" (Traunecker, "las moléculas de cadena simple biespecíficas (Janusins) dirigen los linfocitos citotóxicos sobre células infectadas con VIH" *EMBO J* 10:3655-3659 (1991) y Traunecker "Janusin: nuevo diseño molecular para reactivos biespecíficos" *Int J Cancer Suppl* 7:51-52 /1992)).

El presente invento también proporciona proteínas de fusión de albúmina que comprenden, fragmentos o variantes (incluyendo derivados) de un anticuerpo descrito en la presente o conocido en otra parte en el estado de la técnica. Técnicas estándares conocidas por aquellos con experiencia en el estado de la técnica pueden ser utilizadas para introducir mutaciones en la secuencia nucleotida que codifica una molécula del invento, incluyendo, por ejemplo, mutagénesis dirigida a sitios y mutagénesis mediada por PCR lo cual resulta en sustituciones de aminoácidos. Preferiblemente, las variantes (incluyendo derivados) codifican menos de 50 sustituciones aminoácidas, menos de 40 sustituciones aminoácidas, menos de 30 sustituciones aminoácidas, menos de 25 sustituciones aminoácidas, menos de 20 sustituciones aminoácidas, menos de 15 sustituciones aminoácidas, menos de 10 sustituciones aminoácidas, menos de 5 sustituciones aminoácidas, menos de 4 sustituciones aminoácidas, menos de 3 sustituciones aminoácidas, o menos de 2 sustituciones aminoácidas con relación al dominio HV de referencia, VHCDR1, VHCDR2, VHCDR3, dominio VL, VLCDR1, VLCDR2, o VLCDR3. En realizaciones especificas, las variantes codifican sustituciones de VHCDR3. En una realización preferida, las variantes tienen sustituciones aminoácidas conservadoras en uno o más residuos aminoácidos no esenciales predichos.

Los anticuerpos que se unen a una proteína terapéutica y que pueden corresponder a una porción de proteína terapéutica de una proteína fusión de albúmina pueden ser descritos o especificados en términos de los epítopes o porciones de una proteína terapéutica la cual éstos reconocen o a la cual específicamente se unen. Los anticuerpos que específicamente se unen a una proteína terapéutica o un epítope específico de una proteína terapéutica también pueden ser excluidos. Por lo tanto, el presente invento contempla anticuerpos que específicamente se unen a proteínas terapéuticas, y permite la exclusión de los mismos. En realizaciones preferidas, las proteínas de fusión de albúmina que comprenden al menos un fragmento o variante de un anticuerpo que se une a una proteína terapéutica, une los mismos epítopes que el fragmento o variante sin fusionar de ese anticuerpo mismo.

Los anticuerpos que se unen a una proteína terapéutica y que pueden corresponder una porción de proteína terapéutica de una proteína fusión de albúmina también se pueden describir o especificar en términos de su reactividad cruzada. Se incluyen anticuerpos que no se unen a cualquier otro análogo, ortólogo, u homólogo de una proteína terapéutica. Los anticuerpos que fijan a polipéptidos con al menos 95%, al menos 90%, al menos 85%, al menos 80%, al menos ustedes 75%, al menos 70%, al menos 65%, al menos 60%, al menos 55%, y al menos 50% de identidad de secuencia (calculada utilizando métodos conocidos en el estado la técnica y descritos en la presente) a una proteína terapéutica también se incluyen en el presente invento. En realizaciones específicas, los anticuerpos que se unen a una proteína terapéutica y que pueden corresponder a una porción de proteína terapéutica de una proteína de fusión de albúmina tienen reacción cruzada con homólogos murinos, de rata y/o conejo de proteínas humanas y los correspondientes epítopes de las mismas. Los anticuerpos que fijan a polipéptidos con menos de 95%, menos de 90%, menos de 85%, menos de 80%, menos de 75%, menos de 70%, menos de 65%, menos de 60%, menos de 55%, y menos de 50% de identidad de secuencia (calculada utilizando métodos conocidos en el estado de la técnica y descritos en la presente) a una proteína terapéutica también son incluidos en el presente invento. En una realización específica, la radiactividad cruzada anteriormente descrita es con respecto a cualquier polipéptido antigénico o inmunogénico específico simple, o combinaciones de 2, 3, 4, 5o más polipéptidos antigénicos y/o inmunogénicos específicos divulgados en la presente. En realizaciones preferidas, las proteínas de fusión de albúmina que comprenden al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica, tienen características de radiactividad cruzada similares o sustancialmente idénticas en comparación con el fragmento o variante de ese anticuerpo particular mismo.

Adicionalmente, en el presente invento se incluyen los anticuerpos que fijan polipéptidos codificados por polinucleotidos los cuales hibridizan a un polinucleotido que codifica una proteína terapéutica bajo condiciones de hibridización severas (como se escribe en la presente). Los anticuerpos que se unen a una proteína terapéutica y que pueden corresponder a una porción de proteína terapéutica de una proteína de fusión de albúmina de invento también se pueden describir o especificar en términos de su afinidad de unión a un polipéptido del invento. Afinidades de unión preferidas incluyen aquellas con una constante de disociación o Kd menor que 5 x 10^{-2}M, 10^{-2}M, 5x10^{-3}M, 10^{-3}M, 5X10^{-4}M, 10^{-4}M. Afinidades de unión más preferidas incluyen aquellas con una constante de disociación Kd menor que 5 X10^{-5}M, 10^{-5}M, 5 X 10^{-6}M, 10^{-6}M, 5X10^{-7}M, 10^{7}M, 5M, 10^{-10}M, 5 X10^{-11}M, 10^{-11}M, 5 X 10^{-12}M, 10^{-12}M, 5X10^{-13}M, 10^{-13}M, 5 x10^{-14}M, 10^{-14}M, 5X10^{-15}M, o 10^{-15}M. En realizaciones preferidas, las proteínas de fusión de albúmina que comprenden al menos un fragmento o variante del anticuerpo que fija una proteína terapéutica, tienen una afinidad para una proteína o epítope dado similar a aquella del anticuerpo correspondiente (no fusionado a albúmina) que fija la proteína terapéutica, tomando en cuenta la

valencia en la proteína de fusión de albúmina (comprendiendo al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica) y la valencia del anticuerpo correspondiente.

El invento también proporciona anticuerpos que competitivamente inhiben de la unión de un anticuerpo a un epítope de una proteína terapéutica como se determinó mediante cualquier método conocido en el estado de la técnica para determinar unión competitiva, por ejemplo, los inmunoensayos descritos en la presente. En realizaciones preferidas, el anticuerpo inhibe competitivamente la unión al epítope en al menos 95%, al menos 90%, al menos 85%, al menos 80%, al menos 75%, al menos 70%, al menos 60%, o al menos 50%. En realizaciones preferidas, las proteínas de fusión de albúmina que comprenden al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica, competitivamente inhiben la unión de un segundo anticuerpo a un epítope de una proteína terapéutica. En otras realizaciones preferidas, las proteínas de fusión de albúmina comprendiendo al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica, competitivamente inhiben la unión de un segundo anticuerpo a un epítope de una proteína terapéutica en al menos 95%, al menos 90%, al menos 85%, al menos 80%, al menos 75%, al menos 70%, al menos 60%, o al menos 50%.

Los anticuerpos que se unen a una proteína terapéutica y que puede corresponder a una porción de proteína terapéutica de una proteína de fusión de albúmina del invento, pueden actuar como agonistas o antagonistas de la proteína terapéutica. Por ejemplo, el presente invento incluye anticuerpos que interrumpen las interacciones receptor/ligando con los polipeptidos del invento parcial o totalmente. El invento destaca anticuerpos específicos a receptores y anticuerpos específicos a ligandos. En invento también destaca anticuerpos específicos a receptores los cuales no evitan unión de ligandos pero evitan activación de receptores. La activación de receptores (es decir, señalización) se puede determinar mediante técnicas descritas en la presente o de otra manera conocidas en el estado de la técnica. Por ejemplo, la activación de receptores se puede determinar detectando la fosforilación (ejemplo, tirosina o serina/treonina) del receptor o su sustrato mediante inmunoprecipitación seguida por análisis western blot (por ejemplo, como se describe anteriormente). En realizaciones especificas, se proporcionan anticuerpos que inhiben actividad de ligandos o actividad de receptores en al menos 95%, al menos 90%, al menos 85%, al menos 80%, al menos 75%, al menos 70%, al menos 60%, o al menos 50% de la actividad en ausencia del anticuerpo. En realizaciones preferidas, las proteínas de fusión de albúmina que comprenden al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica, tienen características similares o sustancialmente similares con respecto a la prevención de unión de ligandos y/o prevención de activación de receptores en comparación con una variante o fragmento sin fusionar del anticuerpo que fija la proteína terapéutica.

El invento también destaca anticuerpos específicos a receptores los cuales previenen unión de ligandos y activación de receptores así como también anticuerpos que reconocen el complejo receptor-ligando, y preferiblemente, no reconocen específicamente el receptor sin unir o en ligando sin unir. Igualmente, se incluyen en el invento anticuerpos neutralizantes los cuales unen el ligando y evitan la unión del ligando al receptor, así como también anticuerpos que fijan en ligando, evitando así la activación de receptores, pero no evitan que en ligando se una el receptor. Además se incluyen en el invento anticuerpos que activan el receptor. Estos anticuerpos pueden actuar como agonistas de receptores, es decir, potenciar o activar todas o un subgrupo de actividades biológicas de la activación de receptores mediada por ligandos, por ejemplo, al inducir dimerización del receptor. Los anticuerpos se pueden especificar como agonistas, antagonistas o agonistas inversos para actividades biológicas que comprenden las actividades biológicas específicas de las proteínas terapéuticas (ej., como se divulga en la Tabla 1). Los anteriores agonistas de anticuerpos se pueden preparar utilizando métodos conocidos en el estado

de la técnica. Ver, ej., Publicación PCT WO 96/40281; Patente Americana No. 5.811.097; Deng, Blood 92(6):1981-1988); Chen, Cancer Res. 58(16):3668-3678 (1998); Harrop J. Immunol. 161(4):1786-1794(1998); Zhu, Cancer Res. 58(15):3209-3214(1998); Yoon, J. Immunol. 160(7):3170-3179 (1998); Prat J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard, J. Immunol. Methods 205(2):177-190 (1997); Liautard, Cytokine 9(4):233-241 (1997); Carlson J-Biol. Chem. 272(17):11295-11301 (1997); Taryman, Neuron 14(4):755-762 (1995), Muller, Structure 6(9):1153-1167 (1998); Bartunek, Cytokine 8(1):14-20 (1996) (las cuales se incorporan como referencia en sus totalidades). En realizaciones preferidas, las proteínas de fusión de albúmina que comprenden al menos un fragmento o variante de un anticuerpo que fija una proteína Terapéutica, tienen propiedades agonistas o antagonistas similares o sustancialmente idénticas que un fragmento o variante sin fusionar del anticuerpo que fija la proteína Terapéutica.

Se pueden utilizar anticuerpos que se unen a una proteína terapéutica y que pueden corresponder a una porción de proteína terapéutica de una proteína de fusión de albúmina del invento, por ejemplo, para purificar, detectar, y dirigir proteínas terapéuticas, incluyendo métodos diagnósticos y terapéuticos *in vitro* e *in vivo*. Por ejemplo, los anticuerpos tienen utilidad en inmunoensayos para medir de manera cualitativa y cuantitativa los niveles de la proteína terapéutica en muestras biológicas. Ver, ej., Harlow, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); incorporada en la presente como referencia en su totalidad. Igualmente, se pueden utilizar proteínas de fusión de albúmina que comprenden al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica, por ejemplo, para purificar, detectar, y dirigir proteínas terapéuticas, incluyendo métodos diagnósticos y terapéuticos *in vitro* y *in vivo*.

Anticuerpos que se unen a una proteína terapéutica y que pueden corresponder a una porción de proteína terapéutica de una proteína de fusión de albúmina incluyen derivados que son modificados, es decir mediante unión covalente de cualquier tipo de molécula al anticuerpo. Por ejemplo, pero no a manera de limitación, los derivados de anticuerpos incluyen anticuerpos que han sido modificados, ej., mediante glicosilación, acetilación, pegilación, fosforilación, amidación, derivatización mediante grupos protectores/bloqueadores, y eliminación proteolítica, unión a un ligando celular y otra proteína, etc. Cualquiera entre numerosas modificaciones químicas se puede realizar mediante técnicas conocidas, incluyendo, pero sin limitar a escisión química específica, acetilación, formilación, síntesis metabólica de tunicamicina, etc. Adicionalmente, el derivado puede contener uno o más aminoácidos no clásicos. Las proteínas de fusión de albúmina del invento también se pueden modificar como se describió anteriormente.

Métodos para producir anticuerpos que fijan proteínas terapéuticas

Los anticuerpos que se unen a una proteína terapéutica y que pueden corresponder a una porción de proteína terapéutica de una proteína de fusión de albúmina del invento se pueden generar mediante cualquier método apropiado conocido en el estado de técnica. Anticuerpos policlonales a un antígeno de interes se pueden producir mediante varios procedimientos bien conocidos en el estado de la técnica. Por ejemplo, se puede administrar una proteína terapéutica a varios animales huesped incluyendo, pero sin limitar a conejos, ratones, ratas, etc. para inducir la producción de sueros que contienen anticuerpos policlonales específicos para el antígeno. Se pueden utilizar varios coadyuvantes para aumentar la respuesta inmunológica, dependiendo de la especie huesped, e incluyen pero no están limitados a coadyuvantes de Freund (completos e incompletos), geles minerales tales como hidróxido de aluminio, sustancias tensoactivas tales como lisolecitina, polioles plurónicos, polianiones, péptidos, emulsiones de aceite, hemocianinas keyhole limpet, dinitrofenol, y coadyuvantes humanos potencialmente útiles tales como BCG

(Bacilos Calmette-Guerin) y corynebacterim parvum. Tales coadyuvantes también son bien conocidos en el estado de la técnica.

Los anticuerpos monoclonales se pueden preparar utilizando una variedad de técnicas conocidas en el estado de técnica incluyendo el uso de tecnologías del hibridoma, recombinantes y de presentación de fagos o una combinación de las mismas. Por ejemplo, los anticuerpos monoclonales se pueden producir utilizando técnicas de hibridoma incluyendo aquellas conocidas en el estado de la técnica y enseñadas, por ejemplo, en Harlow, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, en: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981) (dichas referencias se incorporan en la presente en su totalidad). El término "anticuerpo monoclonal" como se utiliza en la presente no está limitado a anticuerpos producidos a través de tecnología de hibridomas. El término "anticuerpo monoclonal" se refiere a un anticuerpo que es derivado a partir de un único clon, incluyendo cualquier clon eucariótico, procariotico, o fago, y no el método mediante el cual es producido.

Los métodos para producción y selección de anticuerpos específicos utilizando tecnología de hibridomas son comunes y bien conocidos en el estado de la técnica. En un ejemplo no limitante, se pueden inmunizar ratones con una proteína terapéutica o fragmento o variante de la misma, una proteína de fusión de albúmina, o una cédula que expresa tal proteína terapéutica o fragmento o variante de la misma o proteína de fusión de albúmina. Una vez detectada la respuesta inmune, ej., los anticuerpos específicos para el antígeno son detectados en el suero de ratón, se cosecha el bazo de ratón y se aislan los esplenocitos. Luego los esplenocitos son fusionados mediante técnicas bien conocidas a cualquier célula de mielanoma apropiada, por ejemplo células de la línea celular SP20 disponibles en ATCC. Los hibridomas son seleccionados y clonados mediante dilución limitada. Luego los clones de hibridomas son probados mediante métodos conocidos en el estado de la técnica para células que secretan anticuerpos capaces de unirse a un polipéptido del invento. La ascitis, la cual generalmente contiene altos niveles de anticuerpos, se puede generar al inmunizar ratones con clones de hibridoma positivos.

Por consiguiente, el presente invento proporciona métodos de generación de anticuerpos monoclonales así comon también anticuerpos producidos mediante el método que comprende el cultivo de una célula de hibridoma que secreta un anticuerpo en donde, preferiblemente, el hibridoma se genera al fusionar esplenocitos aislados a partir de un ratón inmunizado con un antígeno del invento con células de mieloma y luego seleccionando los hibridomas que resultan de la fusión para clones de hibridoma que secretan un anticuerpo capaz de unirse a un polipeptido del invento.

Otro método bien conocido para producir líneas celulares B policlonales y monoclonales humanas es la trasformación utilizando el virus Epstein Barr (EBV). Los protocolos para generar líneas celulares B EBV-transformadas comúnmente son conocidos en el estado de la técnica, tal como, por ejemplo, el protocolo descrito en el Capitulo 7-22 de Protocolos Actuales de Inmunología, Coligan, Eds., 1994, John Wiley & Sons, NY, el cual se incorpora en la presente como referencia en su totalidad. La fuente de células B para transformación comunmente es sangre periférica humana, pero las células B para transformación también se pueden derivar a partir de otras fuentes incluyendo, pero sin limitar a nodos linfáticos, amígdala, bazo, tejido tumoral, y tejidos infectados. Los tejidos generalmente se preparan en suspensiones de células simples antes de transformación EBV. Adicionalmente, se pueden tomar pasos para remover fisicamente o activar células T (ej., mediante tratamiento con ciclosporina A) en muestras que contienen células B, puesto que las células T de inviduos seropositivos para anticuerpos anti-EBV pueden suprimir inmortalización de células B mediante EBV.

En general, la muestra que contiene células B humanas es incubada con EBV, y cultivado durante 3-4 semanas. Una fuente típica de EBV es el sobrenadante de cultivo de la línea celular B95-8 (ATCC #VR-1492). Signos físicos de transformación EBV se pueden observar hacia el final del periodo de cultivo de 3-4 semanas. Mediante microscopia de contraste de fase, las células transformadas pueden aparecer grandes, claras, pilosas y tender a agregarse en haces apretados de células. Las líneas EBV generalmente son policlonales. Sin embargo, durante periodos prolongados de cultivos celulares, las líneas EBV pueden volverse monoclonales o policlonales como resultado del crecimiento externo selectivo de clones de células B particulares. Alternativamente, las líneas EBV transformadas policlonales se pueden ser subclonar (ej., limitando cultivo de dilución) o fusionar con un socio de fusión adecuado y blindados para limitar la dilución para obtener líneas de celulas B monoclonales. Socios de fusión adecuados para líneas celulares EBV-transformadas incluyen líneas celulares de mieloma de ratón (ej., SP2/0, X63-Ag8.653)), líneas celulares de heteromieloma (humano x ratón); ej., SPAM-8, SBC-H20, y CB-F7), y líneas celulares humanas (ej., GM 1500, SKO-007, RPM1 8226, y KR-4). Por lo tanto, el presente invento también proporciona un método para generar los anticuerpos humanos policlonales o monoclonales contra polipéptidos del invento o fragmentos de los mismos, comprendiendo transformación-EBV de células B humanas.

Los fragmentos de anticuerpos que reconocen epítopes específicos se pueden generar mediante técnicas conocidas. Por ejemplo, los fragmentos Fab y F(ab')2 del invento se pueden producir mediante escisión proteolítica de moléculas de inmunoglobulina, utilizando enzimas tales como papaina (para producir fragmentos Fab) o pepsina (para producir fragmentos F(ab')2). Los fragmentos f(ab')2 contienen en la región variable, la región constante de cadena liviana y el dominio CH1 de la cadena pesada.

Por ejemplo, los anticuerpos que se unen a una proteína terapéutica también se pueden generar utilizando varios métodos de presentación de fagos conocidos en el estado de la técnica. Los dominios de anticuerpos funcionales son visualizados sobre la superficie de las partículas de fagos que llevan las secuencias polinucleotidas que los codifican. En una realización particular, tales fagos se pueden utilizar para visualizar dominios de unión de antígenos expresados a partir de un repertorio o biblioteca combinatorial de anticuerpos (ej., humanos o murinos). El fago que expresa un dominio de fijación de antígenos que fija el antígeno de interés se puede seleccionar o identificar con antígeno, ej., utilizando antígeno rotulado o antigeno fijado o capturado sobre una superficie sólida o perla. Los fagos utilizados en estos métodos típicamente son fagos filamentosos incluyendo dominios de unión fd y M13 expresados a partir de fagos con dominios de anticuerpos Fab, Fv o Fv estabilizado con disulfuro recombinantemente fusionados con proteínas del fago del gen III o gen VIII. Ejemplos de métodos de presentación de fagos que se pueden utilizar para preparar anticuerpos que se unen a una proteína terapéutica incluyen aquellos divulgados en Brinkman, J. Immunol. Methods 182-41-50 (1995); Ames, J. Immunol. Methods 184:177-186 (1995); Kettleborough, Eur, J. Immnunol 24:952-958 (1994); Persic, Gene 187 9-18 (1997); Burton, Avances en Inmunología 57:191-280 (1994); solicitud PCT No. PCT/GB91/01134; publicaciones PCT WO 90/02809; WO 91710737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; y Patentes Americanas No. 5.698.426; 5.223.409; 5.403.484; 5.580.717; 5.427.908; 5.750-753; 5.821.047; 5.571.698; 5.427.908; 5.516.637; 5.780.225; 5.658.727; 5.733.743 y 5.969.108; cada una de estas es incorporada en la presente como referencia en su totalidad.

Como se describe en las anteriores referencias, después de la selección de fagos, el anticuerpo que codifica las regiones a partir de los fagos se puede aislar y utilizar para generar anticuerpos enteros, incluyendo anticuerpos humanos, o cualquier otro fragmento de fijación de

antígenos deseado, y expresados en cualquier huésped deseado, incluyendo células mamíferas, células de insectos, células vegetales, levadura y bacterias, ej., como se describe en detalle a continuación. Por ejemplo, también se pueden utilizar técnicas para producir de manera recombinante fragmentos Fab, Fab' y f(ab')2 utilizando métodos conocidos en el estado de la técnica tales como aquellos divulgados en publicación PCT WO 92/22324; Mullinax, BioTechniques 12(6):864-869 (1992); y Sawai, AJRI 34:26-34 (1995); y Better, Science 240:1041-1043 (1988) (dichas referencias se incorporan en la presente como referencia en su totalidad).

Ejemplos de técnicas que se pueden utilizar para producir anticuerpos y Fvs de cadena simple incluyen aquellas descritas en las Patentes Americanas No. 4.946.778 y 5.258.498; Huston, Methods in Enzymology 203:46-88 (1991); Shu, PNAS 90:7995-7999 (1993); y Skerra, Science 240:1038-1040 (1988). Para algunos usos, incluyendo uso *in vivo* de anticuerpos en humanos y ensayos de detección in vitro, puede ser preferible utilizar anticuerpos quiméricos, humanizados, o humanos. Un anticuerpo quimérico es una molecula en la cual diferentes porciones del anticuerpo son derivadas a partir de diferentes especies animales, tales como anticuerpos con un región variable de un anticuerpo monoclonal murino y una región constante de inmunoglobulina humana. Métodos para producir anticuerpos quiméricos son conocidos en el estado de la técnica, Ver ej., Morrison, Science 229:1202 (1985); Oi, BioTechniques 4:214 (1986); Gillies, (1989) J. Immunol Methods 125:191-202; Patente Americana No. 5.807.715; 4.816.567, y 4.816.397, las cuales se incorporan en la presente como referencia en su totalidad. Los anticuerpos humanizados son moleculas de anticuerpos de anticuerpo de especies no humanas que fijan el antígeno deseado con una o más regiones determinadoras de complementariedad (CDRs) a partir de especies no humanas y una región de marco a partir de una molecula de inmunoglobulina. A menudo, los residuos de marco en las regiones de marco humanas serán sustituidos con el correspondiente residuo a partir del anticuerpo donante CDR para alterar, preferiblemente mejorar, unión de antígenos. Estas sustituciones de marco son identificadas mediante métodos conocidos en el estado de la técnica, ej., mediante modelación de las interacciones de la CDR y los residuos de marco para identificar residuos de marco importantes para unión de antígenos y comparación de secuencias para identificar residuos de marco inusuales en posiciones particulares. (Ver, ej., Queen. Patente Americana No. 5.585.089; Riechmann, Nature 332:323 (1988), las cuales se incorporan en la presente como referencia). Los anticuerpos se pueden humanizar utilizando una variedad de técnicas conocidas en el estado de la técnica, incluyendo por ejemplo, injerto-CDR (EP 239.400; publicación PCT WO 91/09967; Patente Americana No. 5.225.539; 5.530.101; y 5.585.089), enchapado o alisamiento (EP 592.106; EP 519.596; Padlan, Molecular Immunology 28(4/5):489-498(1991); Studnicka, Protein Engineering 7(6):805-814 (1994); Roguska, PNAS 91:969-973 (1994)), y entremezclado de cadenas (Patente Americana No. 5.565.332).

Los anticuerpos completamente humanos son particularmente deseables para tratamiento terapéutico de pacientes humanos. Los anticuerpos humanos se pueden preparar mediante una variedad de métodos conocidos en el estado de la técnica incluyendo métodos de presentación de fagos descritos anteriormente utilizando bibliotecas de anticuerpos derivadas de secuencias de inmunoglobulina. Ver tambien Patente Americana No. 4.444.887; y publicación PCT WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, y WO 91/10741; cada una de estas incorporada como referencia en su totalidad.

Los anticuerpos humanos también se pueden producir utilizando ratones transgénicos que no pueden expresar inmunoglobulinas endógenas funcionales, pero pueden expresar genes de inmunoglobulina humana. Por ejemplo, los complejos de genes de inmunoglobulina humna de

cadena pesada y liviana se pueden introducir de manera aleatoria o mediante recombinación homologa en células madre embrionarias de ratón. Alternativamente, la región variable humana, región constante, y región de diversidad se pueden introducir en células madre embrionarias de ratón además de los genes humanos de cadena pesada y liviana. Los genes de inmunoglobulina de cadena pesada y liviana de ratón se pueden hacer no funcionales de manera separada o simultáneamente con la introducción de sitios de inmunoglobulina humana mediante recombinación homóloga. En particular, la deleción homozigótica de la región JH evita la producción de anticuerpos endógenos. Las células madre enbrionarias modificadas son expandidas y microinyectadas en blastocistos para producir los ratones quiméricos. Los ratones quiméricos luego son criados para producir progenie homozigótica la cual expresa anticuerpos humanos. Los ratones transgénicos son inmunizados en la forma normal con un antígeno seleccionado, ej., toda o parte de un polipéptido del invento. Los anticuerpos monoclonales dirigidos contra el antígeno se pueden obtener a partir de ratones inmunizados transgénicos utilizando tecnología de hibridomas convencional. Los transgenes de inmunoglobulina humana contenidos por los ratones transgénicos se redisponen durante diferenciación de células B, y subsecuentemente soportan intercambio de clases y mutación somática. Así, utilizando esta técnica, es posible producir anticuerpos IgG, IgA, IgM y IgEterapeuticamente útiles. Para una perspectiva general de esta tecnología para producir anticuerpos humanos, ver Lonberg y Huszar, Int. Rev. Immnonol. 13:65-93 (1995). Para una discusión detallada de esta tecnología para producir anticuerpos humanos y anticuerpos monoclonales humanos y protocolos para producir tres anticuerpos, ver, ej., PCT publicaciones WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; Europan patent No. 0 598 877; U. S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; 5,939,598; 6,075,181; y 6,114,598, las cuales son incorporadas en la presente como referencia en su totalidad. Además, compañías tales como Abgenix, Inc. (Fremont, Ca) y Genpharm (San Jose, CA) pueden estar comprometidas para proporcionar anticuerpos humanos dirigidos contra un antígeno seleccionado utilizando tecnología similar a la descrita anteriormente.

Anticuerpos completamente humanos los cuales reconocen un epítope seleccionado se pueden generar utilizando la técnica denominada "selección guiada." En este enfoque un anticuerpo monoclonal no humano seleccionado, ej., un anticuerpos de ratón, se utiliza para guiar la selección de un anticuerpo completamente humano que reconoce el mismo epítope. (Jespers, Bio/tecnhonoly 12:899-903 (1988)).

Polinucleotidos que codifican anticuerpos

El invento además proporciona polinucleotidos que comprenden una secuencia nucleotida que codifica un anticuerpo y fragmentos del mismo. El invento también contempla polinucleotidos que hibridizan bajo condiciones severas o alternativamente bajo condiciones de hibridización de severidad menor, ej., como se definió anteriormente, a polinucleotidos que codifican un anticuerpo, preferiblemente, que específicamente se une a una proteína terapéutica, y más preferiblemente, un anticuerpo que se une a un polipéptido con la secuencia de aminoácidos de una "proteína terapéutica:X" como se divulga en la columna "SEQ ID NO:2" de la tabla 2.

Los polinucleotidos se pueden obtener, y la secuencia nucleotida de los polinucleotidos se puede determinar, mediante cualquier método conocido en el estado de la técnica. Por ejemplo, si la secuencia nucleotida del anticuerpo es conocida, un polinucleotido que codifica el anticuerpo se puede ensamblar a partir de oligocleotidos químicamente sintetizados (ej., como se describe en Kutmeier, BioTechniques 17:242 (1994)), los cuales, brevemente, involucran la síntesis de oligocleotidos traslapados que contienen porciones de la secuencia que codifica el anticuerpo,

recocido y ligación de estos oligonucleotidos, y luego amplificación de los oligocleotidos ligados por PCR.

Alternativamente, un polinucleotido que codifica un anticuerpo se puede generar a partir de ácido nucleico desde una fuente adecuada. Si un clon que contiene un ácido nucleico que codifica un anticuerpo particular no esta disponible, pero que se conoce la secuencia de la molécula del anticuerpo, se puede sintetizar y obtener de manera química un ácido nucleico que codifica la inmunoglobulina a partir de una fuente apropiada (ej., una biblioteca cADN de anticuerpos, o una biblioteca cADN generada a partir de, o ácido nucleico, preferiblemente poli A + ARN, aislado de, cualquier tejido o células que expresan el anticuerpo, tales como células de hibridomas seleccionadas para expresar un anticuerpo) mediante amplificación PCR utilizando cebadores sintéticos hibridizables a los extremos 3' y 5' de la secuencia o mediante clonación utilizando sonda oligonucleotida específica para la secuencia genética particular para identificar, ej., un clone de cADN a partir de una biblioteca cADN que codifica el anticuerpo. Luego los ácidos nucleicos amplificados generados mediante PCR se pueden clonar en vectores de clonación replicables utilizando cualquier método bien conocido en el estado de la técnica (ver Ejemplo 65).

Una vez se determina la secuencia nucleotida y la secuencia de aminoácidos correspondiente, la secuencia nucleotida del anticuerpo se puede manipular utilizando métodos bien conocidos en el estado de la técnica para la manipulación de secuencias nucleotidas, ej., técnicas de ADN recombinante, mutagenesis dirigida a sitio, PCR, etc. (ver, por ejemplo, las técnicas descritas en Sambrook, 1990, Clonación Molecular, A Laboratory Manual, 2d Ed. Cold Spring Harbor Laboratory, Cold Spirng Harbor, NY y Ausubel, eds 1998, Current Protocols Biology, John Wiley & Sons, NY, las cuales se incorporan como referencia en su totalidad), para generar anticuerpos que tienen una secuencia de aminoácidos diferente, por ejemplo para crear sustituciones, eliminaciones y/o inserciones de aminoácidos.

En una realizaciones específica, la secuencia de aminoácidos de los dominios variables de cadena liviana y/o pesada se puede inspeccionar para identificar las secuencias de las regiones determinadoras de complementariedad (CDRs) mediante métodos que son bien conocidos en el estado de la técnica, ej., mediante comparación con secuencias de aminoácidos conocidas de otras regiones variables de cadena liviana y pesada para determinar las regiones de hipervariabilidad de secuencia. Utilizando técnicas comunes de ADN recombinante, una o más CDRs se pueden insertar dentro de las regiones de marco, ej., en regiones de marco humanas para humanizar un anticuerpo no humano, como se describió anteriormente. Las regiones de marco pueden ser regiones de marco de ocurrencia natural o consenso, y preferiblemente regiones de marco humanas (ver, ej., Chothia, J. Mol. Biol. 278: 457-479 (1998) para un listado de regiones de marco humanas). Preferiblemente, el polinucleotido generado mediante la combinación de la regiones de marco y CDRs codifica un anticuerpo que específicamente se une a un polipéptido del invento. Preferiblemente, como se discutió anteriormente, una o dos sustituciones aminoácidas se pueden realizar dentro de las regiones de marco, y, preferiblemente, las sustituciones aminoácidas mejoran la unión del anticuerpo a su antígeno. Adicionalmente, tales métodos se pueden utilizar para preparar sustituciones o eliminaciones de aminoácidos de uno o más residuos de cisteina de regiones variables que participan en un enlace de disulfuro dentro de la cadena para generar moléculas de anticuerpos que carecen de uno o más enlaces de disulfuro dentro de la cadena. Otras alteraciones al polinucleotido son contempladas por el presente invento y están dentro de la destreza del estado de la técnica.

Además, se pueden utilizar técnicas desarrolladas para la producción de "anticuerpos quiméricos" (Morrison, Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger, Nature 312:604-

608 (1984); Takeda, Nature 314:452-454 (1985)) al unir genes de una molecula de anticuerpo de ratón de especificidad de antígeno apropiada junto con genes de una molecula de anticuerpo humano de actividad biológica adecuada. Como se definió anteriormente, un anticuerpo quimérico es una molécula en la cual diferentes porciones son derivadas a partir de diferentes especies animales, tal como aquellas que tienen una región variable derivada de un murino mAb y una región constante de inmunoglobulina humana, ej., anticuerpos humanizados.

Alternativamente, las técnicas descritas para producción de anticuerpos de cadena simple (Patente Americana No. 4.946.778; Bird, Science 242:423-42 (1988); Huston, Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); y Ward, Nature 334:544-54 (1989) se pueden adaptar para producir anticuerpos de cadena simple. Los anticuerpos de cadena simple se forman mediante la unión de fragmentos de cadena pesada y liviana de la región Fv a través de un puente aminoácido, resultando en un polipéptido de cadena simple. También se pueden utilizar las técnicas para ensamble de fragmentos Fv funcionales en E. coli (Skerra, Science 242:1038-1041 (1988)).

Expresión recombinante de anticuerpos

La expresión recombinante de un anticuerpo, o fragmento, derivado o análogo del mismo, (ej., una cadena pesada o liviana de un anticuerpo o un anticuerpo de cadena simple), requiere construcción de un vector de expresión que contiene un polinucleotido que codifica el anticuerpo. Una vez obtenido un polinucleotido que codifica una molécula de anticuerpo o una cadena pesada o liviana de un anticuerpo, o porción del mismo (preferiblemente conteniendo dominio variable de cadena pesada o liviana), del invento, el vector para producir a molécula de anticuerpo se puede producir mediante tecnología de ADN recombinante utilizando técnicas bien conocidas en el estado de la técnica. Por lo tanto, los métodos para fabricación de una proteína mediante expresión de un polinucleotido que contiene un anticuerpo que codifica la secuencia nucleotida son descritos en la presente. Los métodos que son bien conocidos por aquellos con experiencia en el estado técnica se pueden utilizar para construir vectores de expresión conteniendo secuencias codificadoras de anticuerpos y señales apropiadas de control de transcripción y traslación. Estos métodos incluyen por ejemplo, técnicas de ADN recombinante in vitro, técnicas sintéticas y recombinación genética *in vivo*. Por lo tanto, el invento proporciona vectores replicables que comprenden la secuencia nucleotida que codifica una molécula de anticuerpo del invento, o una cadena liviano o pesada de la misma, o un dominio variable de cadena pesada o liviana, operablemente unido a un promotor. Tales vectores pueden incluir la secuencia nucleotida que codifica la región constante de la molecula de anticuerpo (ver, ej., Publicación PCT WO 86/05807; Publicación PCT WO 89/01036; y Patente Americana No. 5.122.464) y el dominio variable del anticuerpo se puede clonar en tal vector para expresión de toda la cadena liviana o pesada.

El vector de expresión es transferido a una célula huesped mediante técnicas convencionales y las células transfectadas luego son cultivadas mediante tecnicas convencionales para producir un anticuerpo. Asi, el invento incluye células huesped que contienen un polinucleotido que codifica un anticuerpo del invento, o una cadena pesada o liviana del mismo, o un anticuerpo de cadena simple, operablemente unido a un promotor heterólogo. En realizaciones preferidas para la expresión de anticuerpos de doble cadena, las vectores que codifican las cadenas pesadas y livianas se pueden co-expresar en la célula huesped para expresión de toda la molécula de inmunoglobulina, tal como se detalla a continuación.

Una variedad de sistemas de vectores de expresión y hospederos se pueden utilizar para expresar las moleculas del anticuerpo del invento. Tales sistemas de expresión y hospederos representan vehículos mediante los cuales las secuencias codificadoras de interés se pueden

producir y subsecuentemente purificar, pero también representan células que al ser transformadas o transfectadas con las secuencias codificadoras nucleotidas apropiadas, expresan una molecula de anticuerpo del invento in situ. Estos incluyen pero no están limitados a microorganismos tales como bacterias (ej., E. coli, B. subtilis) transformadas con ADN de bacteriofagos recombinantes, ADN plásmido o ADN cósmido, vectores de expresión que contienen secuencias codificadoras de anticuerpos; levadura (ej., Saccharomyces, Pichia) transformada con vectores de expresión de levadura recombinante que contienen secuencias codificadoras de anticuerpos; sistemas de células de insectos infectadas con vectores de expresión de virus recombinante (ej., virus del mosaico de la coliflor, CaMV; virus del mosaico del tabaco, TMV) o transformados con vectores de expresión de plásmida recombinante (ej., Ti plásmida) conteniendo secuencias codificadoras de anticuerpos; o sistemas de células mamíferas (ej., células COS, CHO, BHK, 293, 3T3) albergando constructos de expresión recombinante que contienen promotores derivados del genoma de células mamíferas (ej., promotor de metalotioneina) o a partir de virus mamíferos (ej., el promotor tardio de adenovirus; el promotor del virus vaccinia 7.5K). Preferiblemente, las células bacterianas tales como E. coli, y más preferiblemente, células eucarioticas, especialmente para la expresión de la molecula de anticuerpo recombinante completo, son utilizadas para la expresión de una molecula de anticuerpo recombinante. Por ejemplo, las células mamiferas tales como células de ovario de hamster Chino (CHO), en conjunción con un vector tal como el elemento promotor de gen temprano intermedio principal a partir de citomegalovirus es un sistema de expresión efectivo para anticuerpos (Foecking, Gene 45:101 (1986); Cockett, Bio/Technology 8:2 (1990)).

En sistemas bacterianos, un numero de vectores de expresión se pueden seleccionar de manera ventajosa dependiendo del uso futuro para la molecula de anticuerpo expresada. Por ejemplo, para la producción de una gran cantidad de tal proteina, para la generación de composiciones farmaceuticas de una molecula de anticuerpo, los vectores que dirigen la expresión de altos niveles de productos de proteinas de fusión que son facilmente purificados, pueden ser deseables. Tales vectores incluyen, pero no están limitados al vector de expresión de E. colo pUR278 (Ruther, EMBO J. 2:1791 (1983)), en el cual la secuencia codificadora de anticuerpos puede estar ligada individualmente en el vector en marco de lectura correcto con la región codificadora lac Z de manera que una proteina de fusión es producida; vectores pIN (Inouye & Inouye, Nucleic Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem. 24:5503-5509 (1989)); y similares. Los vectores pGEX también se pueden utilizar para expresar polipeptidos extranjeros como proteinas de fusión con S-transferasa de glutationa (GST). En general, tales proteinas de fusión son solubles y se pueden purificar facilmente a partir de células lisadas mediante adsorción y unión a perlas de glutationa y agarosa seguido por elución en la presencia de glutationa libre. Los vectores pGEX son diseñados para incluir sitios de escisión por la trombina o por la proteasa factor Xa de manera que el producto genético objetivo clonado puede ser liberado a partir de la fracción de GST.

En un sistema de insectos, el virus de la polihedrosis nuclear de Autographa californica (AcNPV) se utilizó como un vector para expresar genes extranjeros. El virus crece en células *Spodoptera frugiperda*. La secuencia codificadora de anticuerpos puede ser clonada individualmente a regiones no esenciales (por ejemplo de gen de polihedrina) del virus y colocada bajo control de un promotor AcNPV (por ejemplo promotor de polihedrina).

En células huesped mamíferas, un número de sistemas de expresión de base viral pueden ser utilizados. En casos donde se utiliza un adenovirus como un vector de expresión, la secuencia codificadora de anticuerpos de interés puede ser ligada a un complejo de control de transcripción/ traslación de adenovirus, ej., promotor tardio y la secuencia guía tripartita. Luego este gen

quimérico puede ser insertado en el genoma del adenovirus mediante recombinación in vitro o in vivo. La inserción de una región no esencial del genoma viral (ej., E1 o E3) producirá un virus recombinante que es viable y capaz de expresar la molecula de anticuerpo en huéspedes infectados. (ej., ver Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). También se pueden requerir señales específicas de iniciación para traslación eficiente de secuencias codificadoras de anticuerpos insertadas. Estas señales incluyen las secuencias adyacentes y de codon de inicio ATG. Además, el codón de inicio puede estar en fase con el marco de lectura de la secuencia codificadora deseada para asegurar traslación del inserto completo. Estas señales de control traslacional exógeno y codones de iniciación pueden tener una variedad de orígenes, tanto naturales como sintéticos. La eficiencia de expresión puede ser aumentada mediante la inclusión de elementos mejoradores de transcripción apropiados, terminadores de transcripción, etc. (ver Bittmer., Methods in Enzymol. 153-544(1987)).

Además, se puede elegir una cepa de la célula huesped que module la expresión de las secuencias insertadas, o modifique y procese el producto genético en la forma específica deseada. Tales modificaciones (ej., glicosilación) y procesamiento (ej., escisión) de productos protéicos pueden ser importantes para la función de la proteína. Las diferentes células huesped tienen mecanismos característicos y específicos para la modificación y procesamiento post-traslacional de proteínas y productos genéticos. Líneas celulares o sistemas huesped apropiados se pueden elegir para asegurar la modificación correcta y procesamiento de la proteína extraña expresada. Para este fin, se pueden utilizar células huesped eucarióticas las cuales poseen la maquinaria celular para procesamiento adecuado de la transcripción primaria, glicosilación, y fosforilación del producto genético. Tales células huesped mamíferas incluyen pero no están limitadas a CHO, VERY, Hela, COS, MDCK, 293, 3T3, WI38, y en particular, líneas celulares de cáncer de mama tales como, por ejemplo, BT483, Hs578T, HTB2, BT20 y T47D, y línea celular de glándula mamaria normal tal como, por ejemplo, CRL7030 y Hs578Bst.

Para producción de alto rendimiento a largo plazo de proteínas recombinantes, se prefiere expresión estable. Por ejemplo, se pueden diseñar líneas celulares que expresen de manera estable la molécula de anticuerpo. En cambio de utilizar vectores de expresión los cuales contienen orígenes virales de replicación, las células huesped se pueden transformar con ADN controlado mediante elementos de control de expresión apropiados (ej., promotor, mejorador, secuencias, terminadores de transcripción, sitios de poliadenilación, etc.), y un marcador seleccionables. Después de introducción del ADN extranjero, las células modificadas se pueden dejar crecer durante 1-2 días en un medio enriquecido, y luego son conmutadas a un medio selectivo. El marcador seleccionable en el plásmido recombinante confiere resistencia a la selección y permite que las células integren de manera estable el plásmido en sus cromosomas y crezcan para formar focos los cuales a su vez se pueden clonar y expandir a líneas celulares. Este método se puede utilizar de manera ventajosa para diseñar líneas celulares que expresen la molécula de anticuerpo. Tales líneas celulares modificadas pueden ser particularmente útiles para selección y evaluación de compuestos que interactuan directa o indirectamente con la molecula de anticuerpo.

Se puede utilizar un número de sistemas de selección, incluyendo pero sin limitar a timidita quinasa del virus del herpes simplex (Wigler, Cell 11:23 (1977)), y se pueden utilizar genes de fosforilbosiltransferasa de hipoxantina-guanina (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), y fosforibosiltransferasa de adenina en células tk-, hgprt- o aprt, respectivamente. También se puede utilizar resistencia a antimetabolitos como la base de selección para los siguientes genes: dhfr, el cual confiere resistencia a metotrexato (Wigler, Natl, Acad. Sci USA 77:357 (1980); O'Hare, Proc. Acad. Sci. USA 78:1527 (1981)); gpt, el cual confiere resistencia a ácido micofenólico (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072

(1981)); neo, el cual confiere resistencia al aminoglicósido G-418 Clinical Pharmacy 12:488-505; Wu y Wu, Biotheray 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mullingan, Science 260:926-932 (1993); y Morgan y Anderson, Ann. Rev. Biochem. 62:191-217 (1993); Mayo, 1993, TIB TECH 11(5):155-215 (1993)); y hygro, el cual confiere resistencia a higromicina (Santerre, Gen 30:147 (1984)). Métodos comunmente conocidos en el estado de la técnica de técnologia de ADN recombinante se pueden aplicar de manera común para seleccionar el clon recombinante deseado, y tales métodos se describen, por ejemplo, en Ausubel (eds), Current Protocols in Molecular Biology, John Wiley & Sons, NY (!))·); Kriegler, Gene Transfer and Expression, A Laboratory Manual. Stockton Press, NY (1990); y en Capitulos 12 y 13, Dracopoli (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin, J. Mol. Biol. 150:1 (1981), los cuales se incorporan en la presente como referencia en su totalidad.

Los niveles de expresión de una molécula de anticuerpo se pueden aumentar mediante amplificación de vectores (para una reseña, ver Bebbington y Hentschel. El uso de vectores se basa en amplificación genética para la expresión de genes clonados en células mamíferas en clonación de ADN, Vol 3. (Academic Press, Nueva York, 1987)). Cuando un marcador en el sistema de vectores que expresa en anticuerpo es amplificable, el aumento en el nivel de inhibidor presente en cultivo de células huesped aumentará el número de copias del gen marcador. Ya que la región amplificada está asociada con el gen de anticuerpo, la producción del anticuerpo también aumentará (Crouse, Mol. Cell. Biol. 3:257 (1983)).

Los vectores que utilizan glutamina sintasa (GS) o DHFR como los marcadores seleccionables se pueden amplificar en presencia de los fármacos metionina sulfoximina o metotrexato, respectivamente. Una ventaja de los vectores basados en glutamina sintasa es la disponibilidad de líneas celulares (ej., la línea celular de mieloma murino, NSO) las cuales son glutamina sintasa negativas. Los sistemas de expresión de glutamina sintasa también funcionan en células de expresión de glutamina sintasa (ej., Ovario de Hamster Chino (células CHO) proporcionando inhibidor adicional para prevenir el funcionamiento del gen endógeno. Un sistema de expresión de glutamina sintasa y componentes del mismo son detallados en publicaciones PCT: WO87/04462; WO86/05807; WO89/01036; WO89/10404; y WO91/06657 las cuales son incorporadas en la presente como referencia en su totalidad. Adicionalmente, los vectores de expresión de glutamina sintasa que se pueden utilizar según el presente invento están comercialmente disponibles en proveedores, incluyendo, por ejemplo Lonza Biologics, Inc. (Portsmouth, NH). Expresión y producción de anticuerpos monoclonales utilizando un sistema de expresión GS en células de mieloma murino se describe en Bebbington, *Bio/tecnhonology* 10:169(1992) y en Biblia y Robinsson *Biotechnol. Prog* 11:1 (1995) los cuales se incorporan en la presente como referencia.

La célula huésped se puede co-transfectar con dos vectores de expresión del invento, el primer vector que codifica un polipéptido derivado de cadena pesada y el segundo vector que codifica un polipéptido derivado de cadena liviana. Los dos vectores pueden contener marcadores seleccionables idénticos los cuales permiten igual expresión de polipéptidos de cadena pesada y liviana. Alternativamente, se puede utilizar con vector simple el cual codifica, y es capaz de expresar polipéptidos de cadena pesada y liviana. En tales situaciones, la cadena liviana se debe ser colocar antes de la cadena pesada para evitar un exceso de cadena pesada libre de tóxicos (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). Las secuencias codificadoras para las cadenas pesadas y livianas pueden comprender cADN o ADN genómico.

Una vez la molécula de anticuerpo del invento ha sido producida por un animal, se ha sintetizado químicamente, o expresado de manera recombinante, ésta se puede purificar mediante cualquier método conocido en el estado de la técnica para purificación de una molécula de inmunoglobulina, por ejemplo, mediante cromatografía (ej., intercambio iónico, afinidad, particularmente mediante afinidad para el antígeno específico después de Proteina A, y cromatografía por exclusión de tamaños), centrifugación, solubilidad diferencial, o cualquier otra técnica estándar para la purificación de proteínas. Además, los anticuerpos que se unen a una proteína terapéutica y que pueden corresponder a una porción de proteína terapéutica de una proteína de fusión de albúmina del invento o fragmentos de la misma se pueden funcionar a secuencias polipeptídicas heterólogas descritas en la presente o de otra manera conocidas en el estado técnica, para facilitar purificación.

Modificaciones de Anticuerpos

Los anticuerpos que se unen a una proteína terapéutica o fragmentos o variantes se pueden funcionar a secuencias marcadoras, tal como un péptido para facilitar purificación. En realizaciones preferidas, la secuencia aminoácida marcadora es un péptido de hexa-histidina, tal como la etiqueta proporcionada en un vector pQE (QIAGEN, Inc., 9259 Eton Avenue, Chatsworthe, CA, 91311), entre otros, muchos de estos comercialmente disponibles. Como se describe en Gentz , Proc. Natl. Acad. Sci USA 86:821-824 (1989), por ejemplo, hexa-histidina proporciona purificación conveniente de la proteina de fusión. Otras etiquetas peptídicas útiles para purificación incluyen, pero no están limitadas a, la etiqueta de hemaglutinina (también llamada la "etiqueta HA"), la cual corresponde a un epítope derivado de la proteina de hemaglutinina de influenza (Wilson, Cell 37:767 (1984)) y la etiqueta "bandera".

El presente invento además contempla anticuerpos o fragmentos de los mismos conjugados a un agente diagnóstico o terapéutico. Los anticuerpos se pueden utilizar de manera diagnóstica para, por ejemplo, monitorear el desarrollo o avance de un tumor como parte de un procedimiento de prueba clínica para, ejemplo, determinar la eficacia de un régimen de tratamiento dado. La detección se puede facilitar mediante acoplamiento del anticuerpo a una sustancia detectable. Ejemplos de sustancias detectables incluyen varias enzimas, grupos prostéticos, materiales fluorescentes, materiales luminiscentes, materiales bioluminiscentes, materiales radiactivos, metales emisores de positrones que utilizan varias tomografías de emisión de positrones, y iones metálicos paramagnéticos no radiactivos. La sustancia detectable se puede acoplar o conjugar ya sea de manera directa al anticuerpo (fragmento del mismo) o de manera indirecta, a través de un intermedio (tal como por ejemplo, un enlazador conocido en el estado de la técnica) utilizando técnicas conocidas en el estado de la técnica. Ver, por ejemplo, la Patente Americana No. 4.741.900 para iones metálicos los cuales se pueden conjugar a anticuerpos para utilizar como diagnostico según el presente invento. Ejemplos de enzimas apropiadas incluyen peroxidasa de rábano, fosfatasa alcalina, beta-galactosidasa, o acetilcolinesterasa; ejemplos de complejos de grupos prostéticos apropiados incluyen estreptavidina/biotina y avidina/biotina; ejemplos de materiales fluorescentes apropiados incluyen umbeliferona, fluoresceina, isocianato de fluoresceina, rodamina, diclorotriazinilamina fluoresceina, cloruro de dansilo o ficoeritrina; un ejemplo de un material luminiscente incluye luminol; ejemplos de materiales bioluminiscentes incluyen luciferasa, y luciferina, y aequorina; y ejemplos de material radioactivo apropiado incluyen 125I, 131I, 111In o 99Tc. Otros ejemplos de sustancias detectables se han descrito en otras partes en la presente.

Además, un anticuerpo del invento se puede conjugar a una fracción terapéutica tal como una citotoxina, ej., un agente citostático o citocidal, un agente terapéutico un ion metálico radiactivo, ej., emisores alfa tal como, por ejemplo, 213Bi. Una citotoxina o agente citotóxico

incluye cualquier agente es perjudicial para las células. Ejemplos incluyen paclitaxol, citochalasina B, gramicidina D, bromuro de etidio, emetina, mitomicina, etoposido, tenoposido, vincristina, colchicina, doxorubicina, diona de dihidroxi-antracina, mitoxantrona, mitramicina, actinomicina D, 1-dehidrotestosterona, glococorticoides, procaina, tetracaina, lidocaina, propranolol, y puromicina y análogos u homólogos de las mismas. Los agentes terapéuticos incluyen pero no están limitados a antimetabolitos (ej., metotrexato, 6-mercaptopurina, 6-tioguanina, citarabina, decarbacina de 5-fluorouracilo), agentes alquilantes (ej., mecloretamina, tioepa clorambucil, melfalan, carmustina (BSNU) y lomustina (CCNU), ciclotosfamida, busulfan, dibromomanitol, estreptozotocina, mitomicina C, y cis-diclorodiamina platino (II) (DDP) cisplatina), antraciclinas (ej., daunorubicina (anteriormente daunomicina), anticuerpos (ej., dactinomicina (anteriormente actinomicina), bleomicina, mitramicina, y antramicina (AMC), y agentes anti-mitóticos (ej., vincristina y vinblastina).

Los conjugados del invento se pueden utilizar para modificar una respuesta biológica dada, el agente terapéutico o fracción farmacéutica no se considerará limitado a agentes terapéuticos químicos clásicos. Por ejemplo, la fracción farmacéutica puede ser una proteína o polipéptido que posee una actividad biológica deseada. Tales proteínas pueden incluir, por ejemplo, una toxina tal como abrina, ricina A, exotoxina de pseudomonas, o tóxina de difteria; una proteina tal como factor de necrosis tumoral, alfa-interferón, beta-interferón, factor de crecimiento nervioso, factor de crecimiento derivado de plaquetas, activador del plaminógeno tisular, un agente apoptótico, ej., TNF-alfa, TNF-beta, AIM I (Ver, Publicación Internacional No. WO 97/33899), AIM II (Ver, Publicación Internacional No. WO 87/34911), Fas Ligand (Takahashi *Int. Immunol.* 6:1567-1574 (1994)), VEGI (Ver, Publicación Internacional No. WO 99/23105), un agente trombótico o un agente anti-angiogénico, ej., angiostatina o endostatina; o, modificadores de respuesta biológica tales como, por ejemplo, linfoquinas, interleucina-1 ("IL-1"), interleucina-2 ("IL-2"), interleucina-6 ("IL-6"), factor estimulante de colonias de granulocitos y macrófogos ("GM-CSF"), factor estimulante de colonias de granulocitos ("G-CSF"), u otros factores de crecimiento.

Los anticuerpos también se pueden unir a soportes sólidos, los cuales son particularmente útiles para inmunoensayos o purificación del antígeno objetivo. Tales soportes sólidos incluyen pero no están limitados a vidrio, celulosa, poliacrilamida, nailon, poliestireno, cloruro de polivinilo, polipropileno.

Las técnicas para conjugar tal fracción terapéutica a anticuerpos son bien conocidas. Ver, por ejemplo, Arnon, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom, "Antibodies for Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson (eds.), pp.623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies'84: Biological y Applications, Pinchera et al. (eds.), pp, 475-506 (1985); "Analysis, Results, y Future Prospective of the Therapeutic USe Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection y Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), y Thorp et al., "The Preparation y Cytotoxic Properties of Antibody-Toxin Conjugantes", immunol. Rev. 62:119-58 (1982).

Alternativamente, un anticuerpo se puede conjugar a un segundo anticuerpo para formar un heteroconjugado de anticuerpo como lo describe Segal en la Patente Americana No. 4.676.980, la cual se incorpora en la presente como referencia en su totalidad.

Un anticuerpo, con o sin fracción terapéutica conjugada a este, administrado sólo o en combinación con un factor citotóxicos y/o citoquina(s) se puede utilizar como un terapéutico.

Fusión de anticuerpo-albúmina

Los anticuerpos que se unen a una proteína terapéutica y que pueden corresponder a una porción de proteína terapéutica de una proteína de fusión de albúmina del invento incluyen, pero no están limitados a anticuerpos que se unen a una proteína terapéutica divulgada en la columna "proteína terapéutica X" de la tabla 1, o un fragmento o variante de la misma.

En realizaciones específicas, el fragmento o variante de un anticuerpo que inmunoespecíficamente se une a una proteína terapéutica y que corresponde a una porción de proteína terapéutica de una proteína de fusión de albúmina comprende, o alternativamente consta del dominio VH. En otras realizaciones, el fragmento o variante de un anticuerpo que inmunoespecíficamente se une a una proteína terapéutica y que corresponde a una porción de proteína terapéutica de una proteína de fusión de albúmina comprende, o alternativamente consta de una, dos o tres CDRs VH. En otras realizaciones, el fragmento o variante de un anticuerpo que inmunoespecíficamente se une a una proteína terapéutica y que corresponde a una porción de proteína terapéutica de una proteína de fusión de albúmina comprende, o alternativamente consta de la CDR1 VH. En otras realizaciones, el fragmento o variante de un anticuerpo que inmunoespecíficamente se une a una proteína terapéutica y que corresponde a una porción de proteína terapéutica de una proteína de fusión de albúmina comprende o alternativamente consta de la CDR2 VH. En otras realizaciones, el fragmento o variante de un anticuerpo que inmunoespecíficamente se une a una proteína terapéutica y que corresponde a una porción de proteína terapéutica de una proteína de fusión de albúmina comprende o alternativamente consta de la CDR3 VH.

En realizaciones específicas, el fragmento o variante de un anticuerpo que inmunoespecíficamente se une a una proteína terapéutica y que corresponde una porción de proteína terapéutica de una proteína de fusión de albúmina comprende o alternativamente consta del dominio VL. En otras realizaciones, el fragmento o variante de un anticuerpo que inmunoespecíficamente se une a una proteína terapéutica y que corresponde a una porción de proteína terapéutica de una proteína de fusión de albúmina comprende o alternativamente consta de una, dos o tres CDRs VL. En otras realizaciones, el fragmento o variante de un anticuerpo que inmunoespecíficamente se une a una proteína terapéutica y que corresponde a una porción de proteína terapéutica de una proteína de fusión de albúmina comprende o alternativamente consta de la CDR1 VL. En otras realizaciones, el fragmento o variante de un anticuerpo que inmunoespecíficamente se une a una proteína terapéutica y que corresponde a una porción de proteína terapéutica de una proteína de fusión de albúmina comprende o alternativamente consta de la CDR2 VL. En otras realizaciones, el fragmento o variante de un anticuerpo que inmunoespecíficamente se une a una proteína terapéutica y que corresponde a una porción de proteína terapéutica de una proteína de fusión de albúmina comprende o alternativamente consta de la CRD3 VL.

En otras realizaciones, el fragmento o variante de un anticuerpo que inmunoespecíficamente se une a una proteína terapéutica y que corresponde a una porción de proteína terapéutica de una proteína de fusión de albúmina comprende o alternativamente consta de una, dos, tres, cuatro, cinco, o seis CDRs VH y/o VL.

En realizaciones preferidas, el fragmento o variante de un anticuerpo que inmunoespecíficamente se une a una proteína terapéutica y que corresponde a una porción de proteína terapéutica de una proteína de fusión de albúmina comprende, o alternativamente consta de una scFv que comprende el dominio VH del anticuerpo terapéutico, unido al dominio VL del anticuerpo terapéutico mediante un enlazador de péptidos tal como (Gly4Ser)3 (SEQ ID NO:4).

Inmunofenotipificación

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Los anticuerpos del invento o proteínas de fusión de albúmina del invento que comprenden al menos un fragmento o variante de un anticuerpo que se une a una proteína terapéutica (o fragmento o variante de la misma) se pueden utilizar para inmunofenotipicación de líneas celulares y muestras biológicas. Las proteínas terapéuticas del presente invento pueden ser útiles como marcadores específicos a cédulas, o más específicamente como marcadores celulares que son expresados diferencialmente en varias etapas de diferenciación y/o maduración de tipos celulares particulares. Los anticuerpos monoclonales (o proteínas de fusión que comprenden al menos un fragmento o variante de un anticuerpo que se une a una proteína terapéutica) dirigidos contra un epítope específico, o combinación de epítopes, permitirán la clasificación de poblaciones celulares que expresan el marcador. Se pueden utilizar varias técnicas que utilizan anticuerpos monoclonales (o proteínas de fusión de albúmina que comprenden al menos un fragmento o variante de un anticuerpo que se une a una proteína terapéutica) para seleccionar poblaciones celulares que expresan el marcador, e incluir separación magnética utilizando perlas magnéticas recubiertas con anticuerpos, "lavado" con el anticuerpo unido a una matriz sólida (es decir, placa), y citometria de flujo (ver, ej., Patente Americana 5.985.660, *Cell*, 96:737-49 (1999)).

Estas técnicas permiten la selección de poblaciones particulares de células, tales como aquellas que se puede encontrar con malignidades hematológicas (es decir, enfermedad residual mínima (MRD) en pacientes con leucemia aguda) y células "no propias" en trasplantes para prevenir la enfermedad Injerto-versus-Huésped (GVHD, *sigla en inglés*, EI-H). Alternativamente, estas técnicas permiten la selección de células madre hematopoyetico y células progenitoras capaces de experimentar proliferación y/o diferenciación, como se puede apreciar en sangre de cordón umbilical humano.

Caracterización de anticuerpos que se unen a una proteína terapéutica y proteínas de fusión de albúmina que comprenden un fragmento o variante de un anticuerpo que se unen a una proteína terapéutica

Los anticuerpos del invento o proteínas de fusión de albúmina del invento comprendiendo al menos un fragmento o variante de un anticuerpo que se une a una proteína terapéutica (o fragmento o variante de la misma) se pueden caracterizar mediante una variedad de formas. En particular, las proteínas de fusión de albúmina del invento comprendiendo al menos un fragmento o variante de un anticuerpo que se une a una proteína terapéutica se pueden probar en cuanto la capacidad para unirse específicamente a los mismos antígenos específicamente unidos por el anticuerpo que se une a una proteína terapéutica correspondiente al anticuerpo que se une a una porción de proteína terapéutica de la proteína de fusión de albúmina utilizando técnicas descritas en la presente o técnicas modificadoras comunes conocidas en el estado de la técnica.

Los ensayos para evaluar la capacidad de los anticuerpos del invento o proteínas de fusión de albúmina del invento comprendiendo al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica (o fragmento o variante de la misma) para unir (específicamente) una proteína específica o epítope se pueden realizar en solución (ej., Houghten, Bio/Techniques 13:412-421 (1992)), sobre perlas (ej., Lam, Nature 354:82-84 (1991)), en hojuelas (ej., Fodor, Nature 364:555-556 (1993)), sobre bacterias (ej., Patente Americana No. 5.223.409), sobre esporas (ej., Patentes No. 5.571.698; 5.403.484; y 5.223.409), sobre plásmidos (ej., Cull., Proc. Natl. Acad. Sci. USA 89:1865-1869 (1992) o sobre fagos (ej., Scott y Smith, Science 249:386-390 (1990); Devlin, Science 249:404-406 (1990); Cwirla, Proc. Natl. Acad. Sci. USA 87:6378-6382 (1990); y Felici, J. Mol. Biol. 222:301-310 (1991)) (cada una de estas referencias es incorporada en la presente como referencia en su totalidad). Los anticuerpos del invento o proteínas de fusión de albúmina del invento que comprenden al menos un fragmento o variante

de un anticuerpo que fija una proteína terapéutica (o fragmento o variante de la misma) se pueden probar en cuanto a su especificidad y afinidad para una proteína o epítope específico utilizando técnicas modificadoras comunes descritas en la presente o de otra manera conocidas en el estado de la técnica.

Las proteínas de fusión de albúmina del invento comprendiendo al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica se pueden probar para radiactividad cruzada con otros antígenos (ej., moléculas que tienen conservación de secuencia/estructura con la molécula específicamente unida por el anticuerpo que fija una proteína terapéutica (o fragmento o variante de la misma) correspondiente a la porción de proteína terapéutica de la proteína de fusión de albúmina del invento) mediante cualquier método conocido en el estado de la técnica.

Inmunoensayos los cuales se pueden utilizar para analizar fijación (inmunoespecífica) y radiactividad cruzada incluyen, pero están limitados a sistemas de ensayos competitivos y no competitivos utilizando técnicas tales como western blots, radioinmunoensayos, ELISA (ensayo inmunosorbente ligado a enzimas), inmunoensayos tipo "sandwich", ensayos de inmunoprecipitación, reacciones de precipitina, reacciones de precipitina de fusión de gel, ensayos de inmunodifusión, ensayos de aglutinación, ensayos de fijación de complemento, ensayos inmunoradiométricos, inmunoensayos florescentes, inmunoensayos de proteína A, por nombrar solo algunos. Tales ensayos son comunes y bien conocidos en el estado de la técnica (ver, ej., Ausubel, eds, 1994, Current Protocols in Molecular Biology, Vol 1, John Wiley & Sons, Inc., New York, el cual se incorpora en la presente como referencia en su totalidad). Inmunoensayos de ejemplo son descritos brevemente a continuación (pero no a manera de limitación).

Los protocolos de inmunoprecipitación generalmente comprenden lisis de una población de células en amortiguador de lisis tal como amortiguador RIPA (1% NP-40 o Triton X-100, 1% deoxicolato de sodio, 0.1% SDS, 0.15 M NaCl, 0.01 M fosfato de sodio a pH 7.2, 1% Trasylol) suplementado con fosfato de proteinas y/o inhibidores de proteasa (ej., EDTA, PMSF, aprotinina, vanadato de sodio), agregando un anticuerpo del invento o la proteina de fusión de albumina del invento comprendiendo al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica (o fragmento o variante de la misma) al lisado celular, incubando durante un periodo de tiempo (ej., 1 a 4 horas) a 40 grados centigrados, agregando perlas de sefarosa de proteina A y/o proteina G (o perlas recubiertas con un anticuerpo antiidiotípico apropiado o anti-cuerpo anti-albúmina en el caso cuando una proteína de fusión de albúmina que comprende al menos un fragmento o variante de un anticuerpo terapéutico) al lisado celular, incubando durante aproximadamente una hora o más a 40 grados C, lavando las perlas en amortiguador de lisis y resuspendiendo las perlas en el amortiguador de SDS/muestra. La capacidad del anticuerpo o proteína de fusión de albúmina para inmunoprecipitar un antígeno particular se puede evaluar mediante ej., análisis western blot. Alguien con experiencia en el estado de la técnica conocerá cuales parámetros pueden ser modificados para aumentar la unión del anticuerpo o la proteína de fusión de albúmina a un antígeno y disminuir el entorno (ej., pre-eliminación de lisado celular con perlas de sefarosa). Para discusión adicional con respecto a protocolos de inmunoprecipitación ver, ej., Ausubel eds, 1994, Current Protocols in Molecular Biology, Vol 1. John Wiley & Sons, Inc., New York 10.16.1.

El análisis western blot generalmente comprende fabricación de muestras proteicas, electroforesis de las muestras proteicas en un gel de poliacrilamida (ej., 8%-20% SDS-PAGE dependiendo del peso molecular del antígeno), transferencia de la muestra de proteínas desde el gel de poliacrilamida a una membrana tal como nitrocelulosa, PVDF o nailon, bloqueo de la

membrana en solución bloqueadora (ej., PBS con BSA al 3% o leche no grasa), lavado de la membrana en amortiguador de lavado (ej., PBS-Tween 20), aplicación del anticuerpo o proteína de fusión de albúmina del invento (diluida en amortiguador bloqueador) a la membrana, lavado de la membrana en amortiguador de lavado, aplicación de un anticuerpo secundario (el cual reconoce a proteína de fusión de albúmina, ej., un anticuerpo de albúmina sérica anti-humano) conjugado a un sustrato enzimático (ej., peroxidasa de rábano o fosfatasa alcalina) o molecula radioactiva (ej., 32P o 125I) diluida en amortiguador bloqueador, lavando la membrana en amortiguador de lavado, y detectando la presencia del antígeno. Alguien con experiencia en el estado de la técnica conocerá los parámetros que se pueden modificar para aumentar la señal detectada y reducir el ruido de entorno. Para discusión adicional con respecto a los protocolos western blot, ver *ej.*, Ausubel, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York en 10.8.1.

ELISAs comprenden la preparación de antigenos, recubrimiento del pozo de una placa de microtitulos de 96 pozos con el antígeno, lavado del antigeno que no se unió a los pozos, adición del anticuerpo o proteína fusión de albúmina (ej., comprendiendo al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica) del invento conjugada a un compuesto detectable tal como un sustrato enzimático (ej., peroxidasa de rábano o fosfatasa alcalina) a los pozos e incubación durante un período de tiempo, lavando las proteínas de fusión de albúmina no unidas o no específicamente unidas, y detectando la presencia del anticuerpo o proteínas de fusión de albúmina específicamente unidas al antígeno recubriendo el pozo. En ELISAs el anticuerpo o proteína de fusión de albúmina no tiene que ser conjugada a un compuesto detectable; en cambio, un segundo anticuerpo (el cual reconoce la proteína de fusión de albúmina) conjugado a un compuesto detectable puede ser agregado al pozo. Además, en cambio de recubrir pozo con el antígeno, el anticuerpo o la proteína de fusión de albúmina puede ser impregnada al pozo. En este caso, la molécula detectable puede ser el antígeno conjugado a un compuesto detectable tal como un sustrato enzimático (*ej.*, peroxidasa de rábano o fosfatasa alcalina). Alguien con experiencia en el estado de la técnica conocerá cuales parámetros pueden ser modificados para aumentar la señal detectada así como también otras variaciones de ELISAs conocidas en el estado de técnica. Para discusión adicional con respecto a ELISAs ver, Ausubel, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York en 11.2.1.

La afinidad de unión de una proteína de fusión de albúmina a una proteína, antígeno, o epítope y la tasa de disociación de una interacción de proteína de fusión de anticuerpo o albúmina con proteína/antígeno/epítope se puede determinar mediante ensayos de unión competitivos. Un ejemplo de un ensayo de unión competitivo es un radioinmunoensayo que comprende la incubación del antígeno rotulado (ej., 3H o 125I) con el anticuerpo o la proteína de fusión de albúmina del invento en presencia de cantidades crecientes de antígeno no rotulado, y la detección del anticuerpo unido al antígeno rotulado. La afinidad del anticuerpo o proteína de fusión de albúmina para una proteína específica, antígeno, o epítope y las tasas de disociación de fijación se pueden determinar a partir de los datos mediante análisis de Scatchard plot. La competición con una segunda proteína que fija la misma proteína, antígeno o epítope que el anticuerpo o proteína de fusión de albúmina, también se puede determinar utilizando radioinmunoensayos. En este caso, la proteína, antígeno o epítope se incuba con un anticuerpo o proteína de fusión de albúmina conjugada a un compuesto rotulado (ej., 3H o 125I) en la presencia de cantidades crecientes de una segunda proteína sin rotular que fija la misma proteína, antígeno o epítope que la proteína de fusión de albúmina del invento.

En la realización preferida, el análisis cinético BIAcore se utiliza para determinar la unión y las tasas de disociación de proteínas de fusión de albúmina del invento a una proteína, antígeno o epítope. El análisis cinético BIAcore comprende análisis de unión y disociación de anticuerpos, proteínas de fusión de albúmina, o polipéptidos específicos, antígenos o epítopes a partir de hojuelas con polipéptidos específicos inmovilizados, antígenos o epítopes, anticuerpos o proteínas de fusión de albúmina, respectivamente, sobre su superficie.

Usos Terapéuticos

El presente invento además está dirigido a terapias basadas en anticuerpos las cuales involucran administración de anticuerpos del invento o proteínas de fusión de albúmina del invento comprendiendo al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica a un animal, preferiblemente un mamífero, y más preferiblemente un humano, paciente para tratar una o más enfermedades, trastornos, o condiciones divulgadas. Los compuestos terapéuticos del invento incluyen, pero no están limitados a anticuerpos del invento (incluyendo fragmentos, análogos y derivados de los mismos tal como se describen a presente, ácidos nucleicos que codifican el anticuerpo del invento (incluyendo fragmentos, análogos y derivados de los mismos y anticuerpos antiidiotípicos tal como se describen en la presente), proteínas de fusión de albúmina que comprenden al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica, y ácidos nucleicos que codifican tales proteínas de fusión de albúmina. Los anticuerpos del invento o proteína de fusión de albúmina del invento comprendiendo al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica se pueden utilizar para tratar, aliviar o prevenir enfermedades, trastornos, condiciones asociadas con expresión y/o actividad aberrante de una proteína terapéutica, incluyendo, pero sin limitar a cualquiera o más de las enfermedades trastornos, o condiciones descritas en la presente. El tratamiento y/o prevención de enfermedades, trastornos, o condiciones asociadas con expresión y/o actividad aberrante de una proteína terapéutica incluye, pero no está limitado a alivio de los síntomas asociados con estas enfermedades, trastornos o condiciones, los anticuerpos del invento o proteínas de fusión de albúmina del invento comprendiendo al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica se pueden proporcionar en composiciones farmacéuticamente aceptables conocidas en el estado de técnica o descritas en la presente.

En una realización específica y preferida, el presente invento está dirigido a terapias basadas en anticuerpos las cuales involucran administración de anticuerpos del invento o proteínas de fusión de albúmina del invento comprendiendo al menos un fragmento o variante del anticuerpo que fija una proteína terapéutica a un animal, preferiblemente un mamífero, y más preferiblemente un humano, paciente para tratar una o más enfermedades, trastornos, o condiciones, incluyendo pero sin limitar a trastornos neurales, trastornos del sistema inmune, trastornos musculares, trastornos reproductivos, trastornos gastrointestinales, trastornos pulmonares, trastornos cardiovasculares, trastornos renales, trastornos proliferativos, y/o enfermedades y condiciones cancerosas, y/o como se describen en otra parte en la presente. Los compuestos terapéuticos del invento incluyen, pero no están limitados a, anticuerpos del invento (ej., anticuerpos dirigidos a la proteína de longitud total expresada sobre la superficie celular de una célula mamífera; anticuerpos dirigidos a un epítope de una proteína terapéutica y ácidos nucleicos que codifican anticuerpos del invento (incluyendo fragmentos, análogos y derivados de los mismos y anticuerpos antiidiotípicos tal como se describe en la presente). Los anticuerpos del invento se pueden utilizar para tratar, inhibir o prevenir enfermedades, trastornos o condiciones asociadas con actividad y/o expresión aberrante de una proteína terapéutica, incluyendo, pero sin

limitar a cualquiera o más de las enfermedades, trastornos, o condiciones descritas en la presente. El tratamiento y/o prevención de enfermedades, trastornos, o condiciones asociados con expresión y/o actividad aberrante de una proteína terapéutica incluye, pero no está limitado a alivio de los síntomas asociados con estas enfermedades, trastornos o condiciones. Los anticuerpos del invento o proteínas de fusión de albúmina del invento comprendiendo al menos un fragmento variante de un anticuerpo que fija una proteína terapéutica se pueden proporcionar en composiciones farmacéuticamente aceptables conocidas en estado de técnica o descritas en la presente.

Un resumen de las formas en las cuales los anticuerpos del invento o proteínas de fusión de albúmina del invento comprendiendo al menos un fragmento o variante del anticuerpo que fija una proteína terapéutica se pueden utilizar terapeuticamente incluye unión de proteínas terapéuticas de manera local o sistémica en el cuerpo o mediante citotoxicidad directa del anticuerpo, ej. mediada por complemento (CDC) o mediante células efectoras (ADCC). Algunos de estos enfoques se describen a continuación en detalle. Armado con las enseñanzas proporcionadas en la presente, alguien con experiencia del estado de técnica sabrá cómo utilizar los anticuerpos del invento o las proteínas de fusión de albúmina del invento comprendiendo al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica para propósitos de diagnóstico, monitoreo o propósitos terapéuticos sin experimentación indebida.

Los anticuerpo del invento o proteínas de fusión de albúmina del invento comprendiendo al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica se pueden utilizar de manera ventajosa en combinación con otros anticuerpos monoclonales o quiméricos, o con linfoquinas o factores de crecimiento hematopoyetico (tal como, ej., IL-2, IL-3 y IL-7), por ejemplo, los cuales sirven para aumentar el número o actividad de células efectoras que interactúan con los anticuerpos.

Los anticuerpos del invento o proteínas de fusión de albúmina del invento comprendiendo al menos un fragmento variante de un anticuerpo que fija una proteína terapéutica se pueden administrar solos o en combinación con otros tipos de tratamientos (ej., terapia de radiación, quimioterapia, terapia hormonal, inmunoterapia y agentes anti-tumorales). Generalmente, se prefiere la administración de productos con origen en especies o reactividad de especies (el caso de anticuerpos) que sean de la misma especie que el paciente. Así, en una realización preferida, los anticuerpos, fragmentos, derivados, análogos, o ácidos nucleicos humanos, son administrados al paciente humano para terapia o profilaxis.

Se prefiere utilizar anticuerpos inhibidores y/o neutralizantes de alta afinidad y/o potentes *in vivo* contra proteinas Terapeuticas, fragmentos o regiones de las misma, (o el correlato de proteina de fusión de albumina de tal anticuerpo) tanto para inmunoensayos dirigidos a como para terapia de trastornos relacionados con polinucleotidos o polipéptidos, incluyendo fragmentos de los mismos, del presente invento. Tales anticuerpos, fragmentos, o regiones, preferiblemente tendrán una afinidad para polinucleotidos o polipéptidos del invento, incluyendo fragmentos los mismos. Afinidades de unión preferidas incluyen constantes de disociación o Kd menor que 5 x 10^{-2}M, 10^{-2}M, 5x10-^{3}M, 10^{-3}M, 5X10^{-4}M, 10^{-4}M. Afinidades de unión más preferidas incluyen aquellas con una constante de disociación Kd menor que 5 X10^{-5}M, 10^{-5}M, 5 X 10^{-6}M, 10^{-6}M, 5X10^{-7}M, 10^{7}M, 5x10^{-8}M o 10^{-8}M. Afinidades de unión aún más preferidas incluyen aquellas con una constante de disociación o Kd menor que 5x10^{-9}, 10^{-9}M, 5X10-^{10}M 10^{-10}M, 5 X10^{-11}M, 10^{-11}M, 5 X 10^{-12}M, 10^{-12}M, 5X10^{-13}M, 10^{-13}M, 5 x10^{-14}M, 10^{-14}M, 5X10^{-15}M, o 10^{-15}M.

Terapia Genética

En una realización específica, los ácidos nucleicos comprendiendo secuencias que codifican anticuerpos que fijan proteínas terapéuticas o proteínas de fusión de albúmina

comprendiendo al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica son administrados para tratar, inhibir o prevenir una enfermedad o trastorno asociado con expresión y/o actividad aberrante de una proteína terapéutica mediante terapia genética. La terapia genética se refiere a terapia realizada mediante la administración a un sujeto de un ácido expresado o expresible. En esta realización del invento, los ácidos nucleicos producen su proteína codificada que media un efecto terapéutico.

Cualquiera de los métodos para terapia genética disponibles en el estado de la técnica se pueden utilizar según el presente invento.

Demostración de Actividad Terapéutica o Profiláctica

Los compuestos o composiciones farmacéuticas del invento preferiblemente son probados *in vitro*, y luego *in vivo* para la actividad terapéutica o profiláctica, antes de utilizar en humanos. Por ejemplo, los ensayos in vitro para demostrar la utilidad terapéutica o profiláctica de un compuesto o composición farmacéutica incluyen, el efecto de un compuesto sobre una línea celular o una muestra de tejido del paciente. El efecto del compuesto o composición sobre la línea celular y/o muestra de tejido se puede determinar utilizando técnicas conocidas por aquellos con experiencia en el estado de la técnica incluyendo, pero sin limitar a ensayos de formación de roseta y ensayos de lisis celular. Según el invento, los ensyos in vitro que se pueden utilizar para determinar si la administración de un compuesto específico es indicada, incluyen ensayos de cultivos celulares *in vitro* en los cuales se desarrolla una muestra de tejido del paciente en cultivo, y se expone o de otra forma se administra un compuesto, y se observa el efecto de tal compuesto sobre la muestra de tejido.

Administración y Composición Terapeutica/profiláctica

El invento proporciona métodos de tratamiento, inhibición y profilaxis mediante administración a un sujeto de una cantidad efectiva de un compuesto o composición farmacéutica del invento. En una realización preferida, el compuesto es sustancialmente purificado (ej., sustancialmente libre de sustancias que limitan su efecto o producen efectos secundarios indeseados). El sujeto preferiblemente es un animal, incluyendo pero sin limitar a animales tales como vacas, cerdos, caballos, pollos, gatos, perros, etc., y preferiblemente es un mamífero, y más preferiblemente un humano.

Las fórmulaciones y métodos de administración que se pueden emplear cuando el compuesto comprende un ácido nucleico o una inmunoglobulina son descritas anteriormente; fórmulaciones y rutas apropiadas de administración adicionales se pueden seleccionar entre aquellas descritas en la presente a continuación.

Se conocen varios sistemas de liberación y se pueden utilizar para administrar un compuesto del invento, ej., encapsulación en liposomas, micropartículas, microcápsulas, células recombinantes capaces de expresar compuesto, endocitosis mediada por receptores (ver, ej., Wu y Wu, Biol. Chem. 262:4429-4432 (1987)), construcción de un ácido nucleico como parte de un vector retroviral u otro vector. etc. Métodos de introducción incluyen pero no están limitados a rutas intradermales, intramusculares, intraperitoneales, intravenosas, subcutaneas, intranasales, epidurales, y orales. Los compuestos o composiciones se pueden administrar mediante cualquier ruta conveniente, por ejemplo mediante infusión o inyección de bolo, mediante absorción a través de revestimientos epiteliales o mucocutáneos (ej., mucosa oral, rectal o mucosa intestinal, etc.) y se pueden administrar junto con otros agentes biológicamente activos. La administración puede ser sistémica o local. Además, puede ser deseable introducir los compuestos farmacéuticos o composiciones del invento en el sistema nervioso central mediante cualquier ruta apropiada, incluyendo inyección intraventricular e intratecal; la inyección intraventricular se puede facilitar

mediante un catéter intraventricular, por ejemplo, fijado a un depósito, tal como un reservorio Ommaya. También se puede emplear administración pulmonar, ej., mediante el uso de un inhalador o nebulizador, y la fórmulación con un agente en aerosol.

En una realización específica, puede ser deseable administrar los compuestos farmacéuticos o composiciones del invento de manera local al área en necesidad de tratamiento; esto se puede lograr, por ejemplo, y no como limitación, mediante infusión local durante cirugía, aplicación tópica, ej., en conjunción con un vendaje de herida después de cirugía, mediante inyección, por medio de un catéter, por medio de un supositorio, por medio de un implante, dicho implante puede ser de material poroso, no poroso, o gelatinoso, incluyendo membranas, tales como membranas sialásticas, o fibras. Preferiblemente, cuando se administra una proteína, incluyendo un anticuerpo del invento, se debe tener cuidado de utilizar materiales a los cuales la proteína no absorba.

En otra realización, el compuesto o composición se puede liberar en una vesícula, en particular un liposomas (ver Langer, Science 249:1527-1533 (1990); Treat, in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein y Fidler (eds), Liss, Nueva York, pp. 353-365 (1989); Lopez-Berestein, ibid., pp. 317-327; ver generalmente ibid.)

En aún otra realización, el compuesto o composición se puede liberar en un sistema de liberación controlada. En una realización, se puede utilizar una bomba (ver Langer, anteriormente; Seflon, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald, Surgery 88:507 (1980)); Saudek, N. Engl., J. Med. 321:374 (1989)). En otra realización, se pueden utilizarlos materiales poliméricos (ver Aplicaciones Médicas de Liberación Controlada, langer y Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Biodisponibilidad de Fármacos Controlada, Diseño y Desempeño de Productos Farmacéuticos, Smolen y Ball (eds.), Wiley, New York (1984); Ranger y Peppas, J., Macromol. Sic. Rev. Macromol. Chem. 23:61 (1983); ver también Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al ., J. Neurosurg 71:105 (1989)). En otra realización, se puede colocar un sistema de liberación controlada en proximidad de un objetivo terapéutico, ej., el cerebro, requiriendo así una fracción de la dosis sistémica (ver, ej., Goodson, en Medical Applications of Controlled Release, anteriormente, vol 2 pp., 115-138 (1984)).

Otros sistemas de liberación controlada se discuten la reseña de Langer 249:1527-1533 (1990)).

En una realización específica donde el compuesto del invento es un ácido nucleico que codifica una proteína, el ácido nucleico se puede administrar *in vivo* para promover la expresión de su proteína codificada, al construir éste como parte de un vector de expresión de ácido nucleico apropiado y administrarlo de manera que se vuelva intracelular, ej., mediante el uso de un vector de retroviral (ver Patente Americana No. 4.980.286), o mediante inyección directa, o mediante el uso de bombardeo de micropartículas (ej., una pistola de genes; Biolistic, Dupont), o recubriendo con lípidos o receptores de superficie celular o transfectando agentes, o administrando éste en unión con un péptido tipo homeobox (homeosecuencia) el cual se sabe entra al núcleo (ver ej., Joliot, Proc. Natl. Acad. Sci. USA 88:1864.1868 (1991)), etc. Alternativamente, un ácido nucleico se puede introducir de manera intracelular e incorporar dentro del ADN de la célula huesped para expresión, mediante recombinación homóloga.

El presente invento también proporciona composiciones farmacéuticas. Tales composiciones comprenden una cantidad terapeuticamente efectiva de un compuesto, y un vehículo farmacéuticamente aceptable. En una realización específica, el término "farmacéuticamente aceptable" significa aprobado por una agencia reguladora del gobierno Federal o estatal o listado en la Farmacopea estadounidense u otra farmacopea generalmente

reconocida para utilizar en animales, y más particularmente en humanos. El término "vehículo" se refiere a un diluyente, coadyuvante, excipiente, o vehículo con el cual el terapéutico es administrado. Tales vehículos farmacéuticos pueden ser líquidos estériles, tal como agua y aceites, incluyendo aquellos de origen en petróleo, animal, vegetal o sintético, tales como aceite de cacahuate, aceite de soya, aceite mineral, aceite de ajonjolí y similares. El agua es un vehículo preferido cuando la composición farmacéutica se administra de manera intravenosa. También se pueden utilizar soluciones salinas y dextrosa acuosa y soluciones de glicerol como vehículos líquidos, particularmente para soluciones inyectables. Excipientes farmacéuticos apropiados incluyen almidón, glucosa, lactosa, sacarosa, gelatina, malta, arroz, harina, tiza, gel de sílice, estearato de sodio, monoestearato de glicerol, talco, cloruro de sodio, leche descremada en polvo, glicerol, propileno, glicol, agua, etanol y similares. Si se desea, la composición también puede contener cantidades menores de agentes humectantes o emulsificantes o agentes amortiguadores de pH. Estas composiciones pueden tomar la forma de soluciones, suspensiones, emulsiones, tabletas, pastillas, cápsulas, polvos, fórmulaciones de liberación sostenida y similares. La composición se puede fórmular como un supositorio, con aglomerantes y vehículos tradicionales tales como triglicéridos. La fórmulación oral puede incluir vehículos tales como calidades farmacéuticas de manitol, lactosa, almidón, estearato de magnesio, sacarina de sodio, celulosa, carbonato de magnesio, etc. ejemplos de vehículos farmacéuticos apropiados son descritos en "Remington's Pharmaceutical Sciences" de E.W. Martin. Tales composiciones contendrán una cantidad terapeuticamente efectiva del compuesto, preferiblemente en forma pura, junto con una cantidad apropiada de vehículo para proporcionar la forma para administración adecuada al paciente. La fórmulación debe satisfacer el modo de administración.

En una realización preferida, la composición se fórmula según procedimientos comunes como una composición farmacéutica adoptada para administración intravenosa en seres humanos. Típicamente, las composiciones para administración intravenosa son soluciones en amortiguador acuoso isotónico estéril. Donde sea necesario, la composición también puede incluir un agente solubilizante y un anestésico local tal como linocaina para calmar el dolor en el sitio de la inyección. Generalmente, se proporcionan ingredientes de manera separada o mezclados en una forma de dosificación unitaria, por ejemplo, como un polvo liofilizado seco o concentrado libre de agua en un recipiente herméticamente sellado tal como una ampolla o sachette (bolsita) indicando la cantidad de agente activo. Donde la composición es para administrar mediante infusión, ésta se puede dispensar con una botella de difusión que contiene agua o sustancia salina de calidad farmacéutica estéril. Donde la composición se administra mediante inyección, una ampolla de agua estéril para inyección o solución salina se puede proporcionar de manera que los ingredientes se pueden mezclar antes de administración.

Los compuestos del invento se pueden fórmular como formas neutrales o de sal. Sales farmacéuticamente aceptables incluyen aquellas formadas con aniones tales como aquellos derivados de ácidos clorhídrico, fosfórico, acético, oxálico, tartárico, etc., y aquéllos formados con cationes tales como aquellos derivados de sodio, potasio, amonio, cálcio, hidróxidos férricos, isopropilamina, trietilamina, 2-etilamino etanol, histidina, procaina, etc.

La cantidad del compuesto del invento que será efectiva en el tratamiento, inhibición y prevención de una enfermedad o trastorno asociado con expresión y/o actividad aberrante de una proteína terapéutica se puede determinar mediante técnicas clínicas estándares. Además, opcionalmente se pueden emplear ensayos in vitro para ayudar a identificar rangos óptimos de dosificación. La dosis precisa empleada en la fórmulación también dependerá de la ruta de administración, y la severidad de la enfermedad o trastorno, y se debe decidir según el criterio del

médico y las circunstancias de cada paciente. Las dosis efectivas se pueden extrapolar a partir de curvas de dosis-respuesta derivadas a partir de sistemas de prueba de modelos in vitro o animales.

Para anticuerpos, la dosis administrada a un pacientes típicamente está entre 0.1 mg/lg a 100 mg/kg del peso corporal del paciente. Preferiblemente, la dosis administrada a un paciente está entre 0.1 mg/kg y 20 mg/kg del peso corporal del paciente, más preferiblemente entre 1 mg/kg y 10 mg/kg del peso corporal del paciente. Generalmente, los anticuerpos humanos tienen una vida media más prolongada dentro del cuerpo humano que los anticuerpos de otras especies debido a la respuesta inmune a los polipéptidos foráneos. Por lo tanto, dosificaciones menores de anticuerpos humanos y administración menos frecuente es a menudo posible. Además, la dosificación y frecuencia de administración de anticuerpos del invento se puede reducir al aumentar la absorción y penetración de tejidos (ejemplo en el cerebro) de los anticuerpos mediante modificaciones tales como, por ejemplo, lipidación.

Diagnosis e Creación de Imágenes

Anticuerpos rotulados y derivados y análogos de los mismos que fijan una proteína terapéutica (o fragmento o variante de la misma) (incluyendo proteína de fusión de albúmina que comprenden al menos un fragmento o variante de anticuerpos que fijan una proteína terapéutica), se pueden utilizar para propósitos diagnósticos para detectar, diagnosticar, o monitorear enfermedades, trastornos, y/o condiciones asociadas con expresión y/o actividad aberrante de la proteína terapéutica. Este invento proporciona la detección de expresión aberrante de una proteína terapéutica, comprendiendo (a) prueba de la expresión de la proteína terapéutica en células o fluido corporal de un individuo utilizando uno o más anticuerpos específicos al polipéptido de interés y (b) comparación del nivel de expresión genética con un nivel de expresión genética estándar, mediante la cual un aumento o disminución en el nivel de expresión de proteína terapéutica probada en comparación con el nivel de expresión estándar es indicativo de expresión aberrante.

El invento proporciona un ensayo diagnóstico para diagnosticar un trastorno, comprendiendo (a) prueba de la expresión de la proteína terapéutica en células o fluido corporal de un individuo utilizando uno o más anticuerpos específicos a la proteína terapéutica o proteína de fusión de albúmina comprendiendo al menos un fragmento o variante de un anticuerpo específico a una proteína terapéutica, y (b) comparación del nivel de expresión genética con un nivel de expresión genética estándar, mediante la cual un aumento o disminución en el nivel de expresión genética de la proteína terapéutica probada en comparación con el nivel de expresión estándar es indicativo de un trastorno particular. Con respecto al cáncer, la presencia de una cantidad relativamente grande de transcripto (producto de transcripción) en tejido biopsiado de un individuo puede indicar una predisposición para el desarrollo de la enfermedad, o puede proporcionar un medio para detectar la enfermedad antes de la aparición de síntomas clínicos actuales. Una diagnosis más definitiva de este tipo puede permitir a los profesionales de la salud emplear medidas preventivas o tratamiento agresivo temprano previniendo así el desarrollo o progresión adicional del cáncer.

Anticuerpos del invento o proteínas de fusión de albúmina que comprenden al menos un fragmento o variante de un anticuerpo específico a una proteína terapéutica, se pueden utilizar para probar niveles de proteínas en una muestra biológica utilizando métodos inmunohistológicos clásicos conocidos por aquellos con experiencia en el estado de la técnica (ej., ver Jalkanen, J. Cell. Biol. 101:976-985; Jalkanen, J. Cell. Biol. 105:3087-3096 (1987)). Otros métodos basados en anticuerpos útiles para detectar expresión genética de proteínas incluyen inmunoensayos, tal como el ensayo inmunosorbente ligado a enzima (ELISA) y el radioinmunoensayo (RIA). Rótulos de ensayos de anticuerpos apropiados son conocidos en el estado de la técnica e incluyen

rótulos enzimáticos, tales como, glucosa oxidasa; radioisótopos, tales como yodo (^{125}I, ^{121}I), carbono (^{114}C), azufre (^{35}S), tritio (^{3}H), indio (^{112}In), tecnetio (^{99}Tc); rótulos luminiscentes, tales como luminol; y rótulos fluorescentes, tales como fluoresceina y rodamina, y biotina.

Una faceta del invento es la detección y diagnosis de una enfermedad o trastorno asociado con expresión aberrante de una proteína terapéutica en un animal, preferiblemente un mamífero y más preferiblemente un humano. En una realización, la diagnosis comprende: a) administración (por ejemplo, parenteral, subcutanea, o intraperitoneal) a un sujeto de una cantidad efectiva de una molecula rotulada la cual especificamente se une al polipéptido de interés; b) esperar un intervalo de tiempo después de administración para permitir a la molecula rotulada preferencialmente concentrarse en sitios en el sujeto conde la proteina Terapeutica es expresada (y para que las moleculas rotuladas no fijadas sean eliminadas hasta lograr el nivel de entorno); c) determinación del nivel de entorno; y d) detección de la molecula rotulada en el sujeto, tal que la detección de la molecula rotulada por encima del nivel de entorno indica que el sujeto tiene una enfermedad o trastorno particular asociado con expresión aberrante de la proteína terapeutica. El nivel de entorno se puede determinar mediante varios métodos incluyendo, comparación de la cantidad de la molécula rotulada detectada con un valor estándar previamente determinado para un sistema particular.

Se entenderá en el estado de la técncia que el tamaño del sujeto y el sistema de creación de imágenes utilizado determinará la cantidad de fracción de imágenes necesaria para producir imágenes de diagnóstico. En el caso de una fracción de radioisótopo, para un sujeto humano, la cantidad de radioactividad inyectada normalmente oscilará entre 5 y 20 milicuries de 99mTc. El anticuerpo rotulado, fragmento de anticuerpo, o proteína de fusión de albúmina que comprende al menos un fragmento o variante de un anticuerpo que fija una proteína terapéutica, luego preferencialmente se acumulará en una ubicación de las células la cual contiene la proteína terapéutica específica. Se describe la creación de imágenes tumorales in vivo en S.W. Bruchiel, "Inmunofarmacocinética de Anticuerpos Rotulados y sus Fragmentos." (Capítulo 13 en Tumor Imaging: La Detección Radioquímica de Cáncer, S.W. Bruchiel y B.A. Rhodes, eds., Masson Publising Inc. (1982)).

Dependiendo de varias variables, incluyendo el tipo de rotulo utilizado y el modo de administración, el intervalo de tiempo después de administración para permitir a la molécula rotulada concentrarse preferencialmente en sitios en el sujeto y para que las moléculas rotuladas no fijadas sean eliminadas hasta lograr nivel de entorno, está entre 6 y 48 horas o 6 y 24 horas o 6 y 12 horas. En otra realización el intervalo de tiempo después de la administración está entre 5 y 20 días o 5 y 10 días.

En una realización, el monitoreo de la enfermedad o trastorno se realiza mediante repetición del método para diagnosis de la enfermedad, por ejemplo, un mes después de la diagnosis inicial, seis meses después de la diagnosis inicial, un año después de la diagnosis inicial, etc.

La presencie de la molecula rotulada se puede detectar en el paciente utilizando métodos conocidos en el estado de la técnica para escaneo *in vivo*. Estos métodos dependen del tipo de rotulo utilizado. Aquellos con experiencia en el estado de la técnica determinarán el método apropiado para detectar un rótulo particular. Métodos y dispositivos que se pueden utilizar en los métodos diagnósticos del invento incluyen, pero no están limitados a tomografía computarizada (CT), escaneo de cuerpo entero tal como tomografia por emisión de positrones (PET), creación de imágenes por resonancia magnética (MRI), y sonografía.

En una realización específica, la molécula es rotulada con un radioisotopo y es detectada en el paciente utilizando un instrumento quirúrgico sensible a radiación (Thurston, Patente

Americana No. 5.441.050). En otra realización, la molecula es rotulada con un compuesto fluorescente y es detectada en el paciente utilizando un instrumento de escaneo sensible a fluorescencia. En otra realización, la molécula es rotulada con un metal emisor de positrones y es detectada en el paciente utilizando tomografía de emisión de positrones. En aún otra realización, la molecula es rotulada con un rótulo paramagnético y es detectada en un paciente utilizando creación de imágenes por resonancia magnética (MRI). Los anticuerpos que específicamente detectan a proteína de fusión de albúmina pero no albúmina o una proteína terapéutica sola están en una realización preferida. Estos pueden ser utilizados para detectar la proteína de fusión de albúmina como se describe en toda la especificación.

Kits

El presente invento proporciona kits que pueden ser utilizados en los métodos anteriores. En una realización, un kit comprende un anticuerpo, preferiblemente un anticuerpo purificado, en uno o más recipientes. En una realización específica, los kits del presente invento contienen un polipéptido sustancialmente aislado que comprende un epítope el cual es específicamente inmunoreactivo con un anticuerpo incluido en el kit. Preferiblemente, los kits del presente invento además comprenden un anticuerpo de control el cual no reacciona con el polipéptido de interés. En otra realización específica, los kits del presente invento contienen un medio para detectar la unión de un anticuerpo a un polipéptido de interés (ej., el anticuerpo se puede conjugar a un sustrato detectable tal con un compuesto fluorescente, un sustrato enzimático, un compuesto radiactivo o un compuesto luminiscente, o un segundo anticuerpo el cual reconoce el primer anticuerpo y se puede conjugar a un sustrato detectable tal como un compuesto fluorescente, un sustrato enzimático o un compuesto luminiscente, o un segundo anticuerpo el cual reconoce el primer anticuerpo que puede ser conjugado a un sustrato detectable).

En otra realización específica del presente invento, el kit es un kit de diagnóstico para utilizar en selección de suero que contiene anticuerpos específicos contra nucleotidos y polipéptidos proliferativos y/o cancerosos. Tal kit puede incluir un anticuerpo de control que no reacciona con el polipéptido de interés. Tal kit puede incluir un antígeno polipeptídico sustancialmente aislado que comprende un epítope el cual es específicamente inmunoreactivo con al menos un anticuerpo de antígeno anti-polipéptido. Además, tal kit incluye medios para detectar la unión de dicho anticuerpo al antígeno (ej., el anticuerpo se puede conjugar a un compuesto fluorescente tal como fluoresceina o rodamina el cual se puede detectar mediante citometria de flujo). En realizaciones específicas, el kit pueden incluir un antígeno polipeptídico producido manera recombinante o sintetizado de manera química. El antígeno polipeptídico del kit también puede estar ligado a un soporte sólido.

En una realización más específica, el medio de detección del kit anteriormente descrito incluye un soporte sólido al se fija dicho antígeno polipeptídico. Tal kit también pueden incluir un anticuerpo anti-humano no ligado rotulado por reportero. En esta realización, la unión del anticuerpo al antígeno polipeptídico se puede detectar mediante unión de dicho anticuerpo rotulado por reportero.

En una realización adicional, el invento incluye un kit de diagnóstico para utilizar en selección se suero que contiene antígenos del polipéptido del invento. El kit de diagnóstico incluye un anticuerpo sustancialmente aislado específicamente inmunoreactivo con antígenos polipéptidos o polinucleotidos, y medio para detectar la unión del antígeno polinucleotido o polipéptido al anticuerpo. En una realización, el anticuerpo está fijado a un soporte sólido. En una realización específica, el anticuerpo puede ser un anticuerpo monoclonal. El medio de detección del kit puede incluir un segundo anticuerpo monoclonal rotulado. Alternativamente, o además, el

medio de detección puede incluir un antígeno competidor rotulado. En una configuración de diagnóstico, el suero de prueba se hace reaccionar con un reactivo de fase sólida que tiene un antígeno unido a superficie obtenido mediante métodos del presente invento. Después de unión con anticuerpo de antígeno específico al reactivo y retiro de componentes de suero sin unir mediante lavado, y reactivo se hace reaccionar con anticuerpo anti-humano rotulado por reportero para unir el reportero al reactivo en proporción a la cantidad de anticuerpo anti-antígeno unido sobre soporte sólido. El reactivo es lavado nuevamente para retirar el anticuerpo rotulado sin unir, y se determina la cantidad de reportero asociado con reactivo. Típicamente, el reportero es una enzima la cual es detectada al incubar la fase sólida en presencia de un sustrato fluorométrico, luminiscente o colorimétrico apropiado (Sigma, St. Louis, MO).

El reactivo de superficie sólido en el ensayo anterior es preparado mediante técnicas conocidas para fijar material proteico a material de soporte sólido, tal como perlas poliméricas, agitadores embeber (*dip stick*), placa de 96 pozos o material de filtro. Estos métodos de fijación generalmente incluyen absorción no específica de la proteína al soporte o fijación covalente la proteína, típicamente a través de un grupo de amina libre, a un grupo quimicamente reactivo sobre el soporte sólido, tal como un grupo carboxilo, hidróxilo, o aldehido activado. Alternativamente, se pueden utilizar placas recubiertas con estreptomicina en conjunción con antígenos biotinilados.

Por lo tanto, el invento proporciona un sistema de ensayo o lit para realizar este método diagnostico. El kit generalmente incluye un soporte con antígenos ligados a superficie, y un anticuerpo anti-humano rotulado por reportero para detectar anticuerpo-anti-antígeno ligado a superficie.

Proteínas de fusión de albúmina

El presente invento generalmente se relaciona con proteínas de fusión de albúmina y métodos para tratar, prevenir, o aliviar enfermedades o trastornos. Como se utiliza en la presente, "proteína de fusión de albúmina" se refiere a una proteína formada por la fusión de al menos una molécula de albúmina (o un fragmento o variante de la misma) a al menos una molécula de una proteína terapéutica (o fragmento o variante de la misma). Una proteína de fusión de albúmina del invento comprende al menos un fragmento o variante de una proteína terapéutica y al menos un fragmento o variante de albúmina sérica humana, las cuales están asociadas entre sí, preferiblemente mediante fusión genética (es decir, la proteína de fusión de albúmina es generada mediante traslación de un ácido nucleico en el cual un polinucleotido que codifica toda o una parte de una proteína terapéutica es unido el marco de lectura con un polinucleotido que codifica toda o una porción de albúmina). La proteína terapéutica y proteína de albúmina, una vez parte de la proteína de fusión de albúmina, pueden cada una ser conocidas como una "porción", "región" o "fracción" de la proteína de fusión de albúmina.

En una realización preferida, el invento proporciona una proteína de fusión de albúmina codificada por un polinucleotido o constructo de fusión de albúmina descrito en la Tabla 1 o Tabla 2. Los polinucleotidos que codifican estas proteínas de fusión de albúmina también son contemplados por el invento.

Las proteínas de fusión de albúmina del invento, incluyen, pero no están limitadas a proteína de fusión de albúmina codificada por una molécula de ácido nucleico que comprende o alternativamente consta de un polinucleotido que codifica al menos una molécula de albúmina (o fragmento o variante de la misma) unida en marco de lectura a por lo menos un polinucleotido que codifica al menos una molécula de una proteína terapéutica (o fragmento o variante de la misma); una molécula de ácido nucleico que comprende, o alternativamente consta de un

polinucleotido que codifica al menos una molécula de albúmina (o un fragmento o variante de la misma) unida en marco de lectura a por lo menos un polinucleotido que codifica al menos una molécula de la proteína terapéutica (o fragmento o variante de la misma) generada como se describe en la tabla 1, tabla 2 o en los ejemplos; o una molécula de ácido nucleico que comprende o alternativamente consta de un polinucleotido que codifica al menos una molécula de albúmina (o fragmento o variante de la misma) unida en marco de lectura a al menos un polinucleotido que codifica al menos una molécula de una proteína terapéutica (o fragmento variante de la misma), comprendiendo adicionalmente, por ejemplo, uno o más de los siguientes elementos: un vector de auto-replicación funcional incluyendo pero sin limitar a un vector de lanzadera, un vector de expresión, un vector de integración, y/o un sistema de replicación), (2) una región para iniciación de transcripción (ej., una región promotora, tal como por ejemplo, un promotor regulable o inducible, un promotor constitutivo, (3) una región para terminación de transcripción, (4) una secuencia guía, y (5) un marcador seleccionable.

En una realización, el invento proporciona una proteína de fusión de albúmina que comprende o alternativamente consta de una proteína terapéutica (ej., como se describe en la Tabla 1) y una proteína de albúmina sérica. En otras realizaciones, el invento proporciona una proteína de fusión de albúmina que comprende o alternativamente consta de un fragmento biologicamente activo y/o terapeuticamente activo de una proteína terapéutica y una proteína de albúmina sérica. En otras realizaciones, el invento proporciona una proteína de fusión de albúmina que comprende o alternativamente consta de una variante biologicamente activa y/o terapeuticamente activa de una proteína terapéutica y una proteína de albúmina sérica.

En realizaciones adicionales, el invento proporciona una proteína de fusión de albúmina que comprende o alternativamente consta de una proteína terapéutica, y un fragmento biologicamente activo y/o terapeuticamente activo de albúmina sérica. En realizaciones adicionales, el invento proporciona una proteína de fusión de albúmina que comprende o alternativamente consta de una proteína terapéutica y una variante biologicamente activa y/o terapeuticamente activa de albúmina sérica. En realizaciones preferidas, la porción de proteína terapéutica de la proteína de fusión de albúmina es la porción madura una proteína terapéutica.

En realizaciones adicionales, el invento proporciona una proteína de fusión de albúmina que comprende o alternativamente consta de una variante o fragmento biologicamente activo y/o terapeuticamente activo de una proteína terapéutica y un fragmento o variante biologicamente activo y/o terapeuticamente activo de albúmina sérica. En realizaciones preferidas, el invento proporciona una proteína de fusión de albúmina que comprende o alternativamente consta de una porción madura de una proteína terapéutica y la porción madura de albúmina sérica.

Preferiblemente, la proteína de fusión de albúmina comprende HA como la porción N-terminal, y una proteína terapéutica, la porción C-terminal. Alternativamente, también se puede utilizar una proteína de fusión de albúmina que comprende HA como la porción C terminal, y una proteína terapéutica como la porción N-terminal.

En otras realizaciones, la proteína de fusión de albúmina tiene una proteína terapéutica fusionada tanto al extremo-N como al extremo-C de albúmina. En una realización preferida, las proteínas terapéuticas fusionadas en los términos N- y C son las mismas proteínas terapéuticas. En una realización alternativa preferida, las proteínas terapéuticas fusionadas en los términos N y C son proteínas terapéuticas diferentes. En otra realización preferida, las proteínas terapéuticas en los términos N y C son proteínas terapéuticas diferentes las cuales se puede utilizar para tratar o prevenir una enfermedad, trastorno, o condición igual o relacionada (ej., como se lista en la columna "Indicación Preferida Y" de la tabla 1). En otra realización preferida, las proteínas terapéuticas fusionadas en los términos N y C son proteínas terapéuticas diferentes las cuales se

puede utilizar para tratar, aliviar, o prevenir enfermedades o trastornos (ej., como se lista en la columna "Indicación Preferida Y" columna de Tabla 1) las cuales son conocidas en el estado de la técnica por ocurrir comunmente en pacientes de manera simultanea, concurrente, o consecutiva, o las cuales ocurren comunmente en pacientes en asociación entre sí.

Las proteínas de fusión de albúmina del invento contemplan proteínas que contienen una, dos, tres, cuatro, o más moléculas de una proteína terapéutica X dada o variante de la misma fusionada a los terminos -N o -C de una proteina de fusión de albumina del invento, y/o a los terminos N y C de albumina o variante de la misma. Las moleculas de una proteina Terapeutica dada X o variantes de la misma pueden estar en cualquier número de orientaciones, incluyendo, pero sin limitar a una orientación "cabeza a cabeza" (ej., en donde el término N de una molecula de una proteina Terapeutica X es fusionado al N término de otra molecula de la proteina Terapeutica X), o una orientacion "cabeza a cola" (ej., en donde el término C de una molécula de una proteina Terapeutica X es fusionado al extremo N de otra molecula de la proteina terapeutica X).

En una realización, uno, dos, tres, o más polipéptidos de proteina terapeutica X orientados en tandem (o fragmentos o variantes de los mismos) son fusionados a los términos N o C de una proteina de fusión de albumina del invento, y/o a los términos N y/o C de albumina o variante de la misma.

Las proteinas de fusión de albumina del invento además contemplan proteinas que contienen uno, dos, tres, cuatro, o más moleculas de una proteina terapeutica dada X o variante de la misma fusionadas al extremo N o C de una proteina de fusión de albumina del invento, y/o al extremo N y/o C de albumina o variante del mismo, en donde las moleculas son unidas mediante enlazadores peptídicos. Ejemplos incluyen aquellos enlazadores peptídicos descritos en la Patente Americana No. 5.073.627 (incorporada en la presente como referencia). Las proteínas de fusión de albúmina comprenden múltiples polipéptidos de proteina Terapeutica separados mediante enlazadores peptídicos se pueden producir utilizando tecnología de ADN recombinante convencional. Los enlazadores son particularmente importantes cuando se fusiona un péptido pequeño a una molécula HSA grande. El péptido mismo puede ser un enlazador al fusionar copias en tándem del polipéptido con otros enlazadores conocidos se pueden utilizar. Los constructos que incorporan enlazadores son descritos en la Tabla 2 o son aparentes cuando se examina SEQ ID NO:Y.

Además, las proteínas de fusión de albúmina del invento también se pueden producir al fusionar una proteína terapéutica X o variantes de la misma con los términos N y C de albúmina o variantes de la misma en una forma tal para permitir la formación de formas multiméricas intramoleculares y/o intermoleculares. En una realización del invento, las proteínas de fusión de albúmina pueden estar en formas monoméricas o multiméricas (es decir, dímeros, trímeros, tetrámeros y multímeros superiores). En una realización específica, la porción de proteína terapéutica de una proteína de fusión de albúmina esta en forma multimérica (es decir, dímeros, trímeros, tetrámeros y multímeros superiores), en la porción de proteína de albúmina esta en forma monomérica.

Además de la proteína de fusión de albúmina en la cual la porción de albúmina esta N-terminal y C-terminal fusionada de la porción de proteina de albumina, las proteínas de fusión de albúmina del invento también se pueden producir al insertar la proteína terapéutica o péptido de interés (ej., una proteína Terapéutica X como se divulgo en la Tabla 1, o un anticuerpo que fija una proteína Terapeutica o no fragmento o variante de la misma) en una región interna de HA. Por ejemplo, dentro de la secuencia de proteínas de la molécula HA, existe una cantidad de vueltas entre el final y en comienzo de alfa-hélices, las cuales son estabilizadas mediante enlaces

de disulfuro. Las vueltas, determinadas a partir de la estructura de cristal de HA (identificadores PDB 1AO6, 1BJ5, 1BKE, 1BMO, 1E7E a 1E71 y 1UOR) en su mayor parte extendidos lejos del cuerpo de la molécula. Las vueltas son útiles para la inserción, o fusión interna, de péptidos terapeuticamente activos, particularmente aquellos que rerquieren que una estructura secundaria sea funcional, o proteínas terapéuticas, para generar esencialmente una molécula de albúmina con actividad biológica específica.

Las vueltas en estructura de albúmina humana en los cuales los péptidos o polipéptidos se pueden insertar para generar proteínas de fusión de albúmina del invento incluyen: Val54-asn61, THr76-Asp89, Ala92-Glu100, Gln170-Ala176, His 247 - Glu252, Glu 266 - Glu277, Glu 280-His288, Ala362-Glu368, Lys439-Pro447, Val462-Lys475, Thr478-Pro486, y Lys560-Thr566. En realizaciones más preferidas, péptidos o polipéptidos son insertados en las vueltas Val54-Asn61, Gln170-Ala176, y/o Lys560-Thr566 de albúmina humana madura (SEQ ID:1).

Los péptidos a ser insertados se pueden derivar a partir de bibliotecas de presentación de fagos o péptidos sintéticos seleccionados para actividad biológica específica o a partir de las porciones activas de una molécula con la función deseada. Adicionalmente, bibliotecas de péptidos aleatorias se pueden generar dentro de vueltas particulares o mediante inserciones de péptidos aleatorizados en vueltas particulares de la molécula HA y en los cuales se representan todas las posibles combinaciones de aminoácidos.

Tales bibliotecas pueden ser generadas sobre HA o fragmentos de dominio de HA mediante uno de los siguientes métodos:

mutación aleatorizada de aminoácidos dentro de una más vueltas pépticas de HA o fragmentos de dominio HA. Ya sea uno, más o todos los residuos dentro de una vuelta podrían mutar en esta forma;

remmplazo, o inserción en una o más vueltas de HA o fragmentos de dominio HA (es decir, fusión interna) de un peptido aleatorizado de longitud Xn (donde X es un aminoácido y n es el número de residuos;

fusiones de péptidos/proteinas N-, C- o N- y C-terminales además de (a) y/o (b).

El HA o fragmento de dominio HA también se puede hacer multifuncional al insertar péptidos derivados de diferentes selecciones de diferentes vueltas contra diferentes objetivos en la misma HA o fragmento de dominio HA.

En realizaciones preferidas, los péptidos insertados en una vuelta de albúmina sérica humana son fragmentos peptídicos o variantes peptídicas de las proteínas terapéuticas divulgadas en la Tabla 1. Más particularmente, el invento contempla proteínas de fusión de albúmina las cuales comprenden fragmentos peptídicos o variantes peptídicas de al menos siete, al menos ocho, al menos nueve, al menos 10, al menos 11 y, al menos 12, al menos 13, al menos 14, al menos 15, al menos 20, al menos 25, al menos 30, al menos 35, o al menos 40 aminoácidos en longitud insertados en una vuelta de albúmina sérica humana. El invento también contempla proteínas de fusión de albúmina las cuales comprenden fragmentos peptídicos o variantes peptídicas de al menos 7, al menos ocho, al menos nueve, al menos 10, al menos 11, al menos 12, al menos 13, al menos 14, al menos 15, al menos 20, al menos 25, al menos 30, al menos 35, o al menos 40 aminoácidos fusionados al extremo N de albúmina sérica humana. El invento también contempla proteínas de fusión de albúmina las cuales comprenden fragmentos peptídicos o variantes peptídicas del al menos 7, al menos 8,al menos 9, al menos 10, al menos 11, al menos 12, al menos 13, al menos 14, al menos 15, al menos 20, al menos 25, al menos 30, al menos 35, o al menos 40 aminoácidos fusionados al extremo C de albúmina sérica humana. Por ejemplo, los péptidos cortos descritos en la Tabla 1 y 2 (ej., Terapéutico Y) se pueden insertar en vueltas de albúmina.

Generalmente, las proteínas de fusión de albúmina del invento pueden tener una región HA-derivada y una región derivada de proteína Terapéutica. Sin embargo, múltiples regiones de cada proteína se pueden utilizar para preparar una proteína de fusión de albumina del invento. De manera similar, más de una proteína terapéutica se puede utilizar para preparar una proteína de fusión de albumina del invento. Por ejemplo, una proteína Terapéutica se puede fusionar a ambos extremos N- y C-terminales de HA. En tal configuración, las porciones de proteína terapéutica pueden ser las mismas o diferentes moléculas de proteínas Terapéuticas. La estructura de proteínas de fusión de albúmina bifuncionales se puede representar como: X-HA-Y o Y -HA-X.

Por ejemplo, una fusión scFv-HA-1FNbalfa-2b anti-BLyS se puede preparar para modular la respuesta inmune a IFNalfa-2b mediante scFv anti-BLyS. Una alternativa es preparar un dosis bi-funcional (o aún multifuncional) de fusiones HA ej., fusión HA-IFNalfa-2b mezclada con fusión scFv de HA-antiBLyS u otras fusiones HA en varias proporciones dependiendo de función, vida media, etc.

Las proteínas de fusión de albúmina bi- o multifuncionales también se pueden preparar para dirigir la porción de proteína terapéutica a un tipo de celula u órgano objetivo a través de la proteína o péptido en el término opuesto de HA.

Como una alternativa a la fusión de moléculas terapeuticas conocidas, los péptidos se pueden obtener al seleccionar bibliótecas construidas como fusiones a los extremos N-, C- o N y C- de HA, o fragmento de dominio de HA, de típicamente 6, 8, 12, 20 o 25 o Xn (donde X es un aminoácido (aa) y n igual al número de residuos) aminoácidos aleatorizados, y en los cuales todas las combinaciones posibles de aminoácidos fueron representadas. Una ventaja particular de este enfoque es que los péptidos se pueden seleccionar sobre la molécula HA y las propiedades del péptido serían tal como se seleccionaron en lugar de, potencialmente, modificadas como podría ser el caso para un péptido derivado por cualquier otro método.

Adicionalmente, las proteínas de fusión de albúmina del invento pueden incluir un péptido enlazador entre las porciones fusionadas para proporcionar mayor separación física entre las fracciones y así maximizar la accesibilidad de la porción de proteína terapéutica, por ejemplo, para unir a su receptor cognado. El péptido enlazador puede constar de aminoácidos tal que éste sea flexible o más rígido.

La secuencia enlazadora puede ser escindible mediante una proteasa o quimicamente para producir la fracción relacionada con la hormona de crecimiento. Preferiblemente, la proteasa es aquella la cual es producida naturalmente por el huésped, por ejemplo la proteasa kex2 de *S. cerevisae* o proteasas equivalentes.

Por lo tanto, como se describió anteriormente, las proteínas de fusión de albúmina del invento pueden tener la siguiente fórmula R1-L-R2; R2-L-R1; o R1-L-R2-L-R1, en donde R1 es al menos una proteína Terapeutica, secuencia péptida o polipéptida, y no necesariamente la misma proteína Terapéutica, L es un enlazador y R2 es una secuencia de albúmina sérica.

En realizaciones preferidas, las proteínas de fusión de albumina del invento que comprenden una proteína Terapéutica tienen una estabilidad de plama superior en comparación con la estabilidad plasmática de la misma proteína terapéutica cuando no está fusionada a albumina. La estabilidad de plama típicamente se refiere al periodo de tiempo entre la administración de la proteína terapéutica in vivo y su transporte hacia la corriene sanguinea y el momento en el cual la proteína es degradada y eliminada de la corriente sanguinea hacia un órgano tal como el riñón o higado, que finalmente elimina la proteina Terapeutica del cuerpo. La estabilidad plasmática se calcula en términos de vida media de la proteína terapéutica en la corriente sanguinea. La vida media de la proteína terapéutica en la corriente sanguínea se puede determinar fácilmente mediante ensayos comunes conocidos en el estado de la técnica.

En realizaciones preferidas, las proteínas de fusión de albúmina del invento que comprenden una proteína terapéutica tienen una vida en almacenamiento extendida en comparación con la misma proteína terapéutica cuando no está fusionada a albúmina. La vida en almacenamiento típicamente se refiere al periodo de tiempo durante el cual la actividad terapéutica de una proteína terapéutica en solución o en alguna otra fórmulación de almacenamiento, es estable sin pérdida indebida de actividad terapéutica. Muchas de las proteínas terapéuticas son altamente inestables en su estado sin fusionar. Como se describe a continuación, la vida en almacenamiento típica de estas proteínas terapéuticas es marcadamente prolongado al incorporar en la proteína de fusión de albúmina del invento.

Las proteínas de fusión de albúmina del invento con vida en almacenamiento "prolongada" o "extendida" exhiben mayor actividad terapéutica con relación a una estándar que ha sido sometida a las mismas condiciones de almacenamiento y manejo. La estándar puede ser la proteína terapéutica de longitud completa sin fusionar. Cuando la porción de proteína terapéutica de la proteína de fusión de albúmina es un análogo, una variante, o de otra forma es alterada o no incluye la secuencia completa de esa proteína, la prolongación de la actividad terapéutica se puede comparar alternativamente con el equivalente sin fusionar de ese análogo, variante, péptido alterado o secuencia incompleta. Como un ejemplo, una proteína de fusión de albúmina del invento puede retener más de 100% de actividad terapéutica aproximadamente, o más de 105%, 110%, 120%, 130%, 150% o 200% de la actividad terapéutica de una estándar cuando se somete a las mismas condiciones de almacenamiento y manejo que la estándar cuando se comparan en un punto de tiempo dado.

La vida en almacenamiento también se puede evaluar en términos de actividad terapeutica restante después de almacenamiento, normalizada a actividad terapéutica cuando el almacenamiento comenzó. Las proteínas de fusión de albumina del invento con vida en almacenamiento prolongada o extendida como se exhibió mediante la actividad terapeutica prolongada y extendida puede retener más de 50% aproximadamente de actividad terapeutica, 60%, 70%, 80%, o 90% o más de actividad terapéutica de la proteína terapeutica equivalente sin fusionar cuando se somete a las mismas condiciones.

Expresión de las Proteínas de Fusión

Las proteínas de fusión de albumina del invento se pueden producir como moléculas recombinantes mediante secreción a partir de levadura, un microorganismo tal como un bacterium, o una línea celular humana o animal. Preferiblemene, el polipéptido es secretado a partir de las células huésped.

Una realización particular del invento comprende un constructo de ADN que codifica la secuencia de señal efectiva para dirigir secreción en levadura, particularmente una secuencia de señal derivada de levadura (especialmente una que es homóloga al huésped de levadura), y en la molécula fusionada del primer aspecto del invento, no existe una pro-secuencia derivada de levadura entre la señal y el polipéptido maduro.

La señal invertasa del *Saccharomyces cerevisiae* es un ejemplo preferido de una secuencia de señal derivada de levadura.

No se contemplan conjugados de la clase preparada por Poznansky (FEBS Lett. 239:18 (1988)), en los cuaeles los polipéptidos preparados de manera separada son unidos mediante entrecruzamiento químico.

El presente invento también incluye una célula, preferiblemente una célula de levadura transformada para expresar una proteína de fusión de albumina del invento. Además de las células huésped transformadas así mismas, el presente invento también contempla un cultivo de

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estas células, preferiblemente un cultivo monoclonal (clonalmente homogéneo), o un cultivo derivado de un cultivo monoclonal, en un medio nutriente. Si el polipéptido es secretado, el medio contendrá el polipéptido, con las células, o sin las células si han sido filtradas o centrifugadas. Muchos sistemas de expresión son conocidos y se pueden utilizar, incluyendo bacterias (por ejemplo *E. coli* y *Bacilus subtilis*), levaduras (por ejemplo *Saccharomyces cerevisiae, Kluyveromyces lactis* y *Pichia pastoris,* hongos filamentosos (por ejemplo *Aspergillus*), células vejetales, células animales y células de insectos.

Las células de levaduras preferidas para utilizar en la producción de proteínas de fusión de albúmina son D88, DXY1 y BXP10. D88 [leu2-3, leu2-122, canI, praI, ubc4] es un derivado de la cepa precursora AH22*his*$^+$ (también conocida como DB1; ver, *ej* Sleep. Biotecnology 8:42-46 (1990)). La cepa contiene una mutación leu2 la cual permite la selección auxotrópica de 2 plásmidos basados en micras que contienen el gen LEU2. D88 también exhibe una depresión de PRB1 en exceso de glucosa. El promotor PRB1 normalmente controlado por dos puntos de chequeo que monitorean los niveles de glucosa y etapa de crecimiento. El promotor es activado en levadura tipo silvestre ante disminución de glucosa y entrada en fase estacionaria. El gen PRA1 codifica una proteasa vacuolar de levadura, YscA endoproteasa A, que está localizada en el ER. El gen UBC4 está en la ruta de ubiquitinación y está en la tarea de ubicar proteínas de corta vida y anormales para degradación dependiente de ubiquitina. Se encontró que el aislamiento de esta mutación ubc4 aumenta el número de copias de un plásmido de expresión en la célula y produce un nivel aumentado de expresión de una proteína deseada expresada a partir del plásmido (ver, ej., Publicación Internacional No. WO99/00504, incorporada en la presente como referencia en su totalidad).

DXY1, un derivado de D88, tiene el siguiente genotipo: [leu2-3, leu2-122, can1, pra1, ubc4, ura3::yap3]. Además de las mutaciones aisladas en D88, esta cepa también tiene un nocaut (función de un gen eliminado por ingeniería genética) de la proteasa YAP3. Esta proteasa produce escisión de residuos principalmente di-básicos (RR, RK, KR, KK) pero también puede promover la escisión en residuos básicos simples en proteínas. El aislamiento de esta mutación yap3 resultó en niveles superiores de producción HSA de longitud completa (ver, ej., Patente Americana No. 5.965.386 y Kerry Williams, Yeast 14:161-169 (1998), incorporadas en la presente como referencia).

BXP10 tiene el siguiente genotipo: *leu2-3, leu2-122, can1, pra1, ubc4, ura3, yap3::URA3, lys2, hsp150::LYS2, pmt1::URA3.* Además de las mutaciones aisladas en DXY1, está cepa también tiene un knockout del gen PMT1 y el gen HSP150. El gen PMT1 es un miembro de la familia evolucionariamente conservada de dolicil-fosfato-D-manosa proteina O-mannosiltransferasas (Pmts). La topología de transmembrana de Pmt1 indica que ésta es una proteína de membrana integral del retículo endoplásmico con un papel en glicosilación O-ligada. Esta mutación sirve para reducir/eliminar glicosilación O-ligada de fusiones HSA (ver, ej., Publicación Internacional No. WO00/44772, incorporada como referencia en su totalidad). Los estudios revelaron que la proteína Hsp150 es separada ineficientemente de rHA mediante cromatografía de intercambio iónico. La mutación en el gen HSP150 retira el contaminante potencial que tiene dificultad probada para retirar mediante técnicas de purificación estándares. Ver, ej., Patente Americana No. 5.783.423, la cual es incorporada en la presente como referencia en su totalidad.

La proteína deseada es producida en formas convencionales, por ejemplo a partir de una secuencia codificadora insertada en el cromosoma huesped o sobre un plásmido libre. Las cepas son transformadas con una secuencia codificadora para la proteína deseada en cualquiera de las formas usuales, por ejemplo electroporación. Métodos para transformación de levadura mediante

electroporación son divulgados en Becker & Guarente (1990) *Methods Enzymol.* 194, 182.

Células exitosamente transformadas, *es decir*, células que contienen un constructo de ADN del presente invento, se pueden identificar mediante técnicas bien conocidas. Por ejemplo, células que provienen de la introducción de un constructo de expresión pueden ser cultivadas para producir el polipéptido deseado. Las células se pueden cosechar y someter a lisis y su contenido de ADN se examina para la presencia de ADN utilizando un método tal como aquel descrito por Southern (1975) *J. Mol. Biol.* 98, 503 o Berent (1985) *Biotech* 3, 208. Alternativamente, la presencia de la proteína en el sobrenadante se puede detectar utilizando anticuerpos.

Vectores plásmidos de levadura útiles incluyen pRS403-406 y pRS413-416 y generalmente están disponibles en Stratagene Cloning Systems, La Jolla, CA 92037, USA. Los plásmidos pRS403, pRS404, pRS495 y pRS406 son plásmidos Integradores de Levadura (Ylps) e incorporan los marcadores seleccionables de levadura HID3, 7RP1, LEU2 y URA3. Los plásmidos pRS413-416 son plásmidos centroméricos de Levadura (Ycps)

Vectores preferidos para preparar proteinas de fusión para expresión en levadura incluyen pPPC005, pScCHSA, pScNHSA, y pC4:HSA los cuales son descritos en detalle en el Ejemplo 1. La figura 2 muestra un mapa del plásmido pPPC0005 que se puede utilizar como el vector base en el cual los polinucleotidos que codifican las Proteínas Terapéuticas se pueden clonar parar formar fusiones HA. Este contiene un promotor *PRB1 S.cerevisae* (PRB1p) una secuencia guía de fusión (FL), ADN codificando albúmina humana HA (rHA) y una secuencia terminadora de *ADHI S. cerevisae*. La secuencia de la secuencia guía de fusión consta de los primeros 19 aminoácidos del péptido de señal de albúmina sérica humana (SEQ ID NO:3) y los cinco últimos aminoácidos del promotor alfa 1 del factor de conjugación (SLDKR, ver EP-A-387 319 el cual se incorpora en la presente como referencia en su totalidad).

Los plásmidos pPPC0005, PScCHSA, pScNHSA, y pC4:HSA fueron depositados en Abril 11 de 2001 en la American Type Culture Collection, 10801 Boulevard University, Manassas, Virginia 20110-2209 y números de acceso dados ATCC PTA-3276, PTA-3279, y PTA-3277, respectivamente. Otro vector útil para expresar una proteína de fusión de albumina en levadura el vector pSAC35 se describe en Sleep, BioTechnology 8:42 (1990) incorporado en la presente como referencia en su totalidad.

Un promotor de levadura que se puede utilizar para expresar la proteína de fusión de albumina es el promotor MET25. Ver, por ejemplo, Dominik Mumburg, Rolf Muller y Martin Funk. Nuclei Acids Research, 1994, Vol. 22, No. 25, pp. 5767-5768. El promotor Met25 tiene 383 bases de longitud (bases -382 a -1) y los genes expresados por este promotor también se conocen como Met15, Met17, y YLR303W. Una realización preferida utiliza la siguiente secuencia, donde, en el extremo 5' de la secuencia a continuación, el sitio No. 1 utilizado en la clonación es está subrayado y el extremo 3' en el codon de inicio ATG está subrayado:

GCGGCCGCCGGAYGCAAGGGTTCGAATCCCTTAGCTCTCATTATTTTTTTGCTTTTTT
CTCTTGAGGTCACATGATCGCAAAATGGAAATGGCACGTGAAGCTGTCGATATTGGG
GGAACTGTGGTGGTTGGCAAATGACTAATTAAGTTAGTCAAGGCGCCATCCTCATGA
AAACTGTGTAACATAATAACCGAAGTGTCGAAAAGGTGGCACCTTGTCCAATTGAAC
ACGTCGATGAAAAAAAATAAGATATATATAAGGTTAAGTAAAGCGTCTGTTAGAAA
GGAAGTTTTTCCTTTTTCTTGCTCTCTTGTCTTTTCATCTACTATTTCCTTCGTGTAATA
CAGGGTCGTCAGATACATAGATACAATTCTATTACCCCTCCATAATG (SEQ ID NO:5)

Promotores adicionales que se pueden utilizar para expresar la proteína de fusión de albúmina en levadura incluyen los siguientes:

a) el promotor cbh1:

TCTAGAGTTGTGAAGTCGGTAATCCCGCTGTATAGTAATACGAGTCGCATCTAAATA
CTCCGAAGCTGCTGCGAACCCGGAGAATCGAGATGTGCTGGAAAGCTTCTAGCGAG
CGGCTAAATTAGCATGAAAGGTATGAGAAATTCTGGAGACGGCTTGTTGAATCATGG
CGTTCCATTCTTCGACAAGCAAAGCGTTCCGTCGCAGTAGCAGGCACTCATTCCCGA
AAAAACTCGGAGATTCCTAAGTAGCGATGGAACCGGAATAATATAATAGGCAATAC
ATTGAGTTGCCTCGACGGTTGCAATGCAGGGGTACTGAGCTTGGACATAACTGTTCC
GTACCCCACCTCTTCTCAACCTTTGGCGTTTCCCTGATTCAGCGTACCCGTACAAGTC
GTAATCACTATTAACCCAGACTGACCGGACGTGTTTGCCCTTCATTTGGAGAAATAA
TGTCATTGCGATGTGTAATTTGCCTGCTTGACCGACTGGGGGCTGTTCGAAGCCCGA
ATGTAGGATTGTTATCCGAACTCTTGCTCGTAGAGGATGTTGTGAATCTGTGTCGGGC
AGGACACGCCTCGAAGGTTCACGGCAAGGGAAACCACCGATAGCAGTGTCTAGTAG
CAACCTGTAAAGCCGAATGCAGCATCACTGGAAAATACAAACCAATGGCTAAAAGT
ACATAAGTTAATGCCTAAAGAAGTCATATACCAGCGGCTAATAATTGTACAATCAAG
TGGCTAAACGTACCGTAATTTGCCAACGGCTTGTGGGGTTGCAGAAGCAACGGCAAA
GCCCCACTTCCCCACGTTTGTTTCTTACTCAGTCCAATCTCAGCTGGTGATCCCCCAA
TTGGGTCGCTTGTTTGTTCCGGTGAAGTGAAGAAGACAGAGGTAAGAATGTCTGACT
CGGAGCGTTTTGCATACAACCAAGGGCAGTGATGGAAGACAGTGAAATGTTGACAT
TCAAGGAGTATTTAGCCAGGGATGCTTGAGTGTATCGTGTAAGGAGGTTTGTCTGCC
GATACGACGAATACTGTATAGTCACTTCTGGTGAAGTGGTCCATATTGAAATGTAAG
TCGGCACTGAACAGGCAAAAGATTGAGTTGAAACTGCCTAAGATCTCGGGCCCTCGG
GCCTTCGGCCTTTGGGTGTACATGTTTGTGCTCCGGGCAAATGCAAAGTGTGGTAGG
ATCGAACACACTGCTGCCTTACCAAGCAGCTGAGGGTATGTGATAGGCAAATGTTCA
GGGGCCACTGCATGGTTTCGAATAGAAAGAGAAGCTTAGCCAAGAACAATAGCCGA
TAAAGATAGCCTCATTAAACGGAATGAGCTAGTAGGAAAGTCAGCGAATGTGTATA
TATAAAGGTTCGAGGTTCCGTGCCTCCCTCATGCTCTCCCCATCTACTCATCAACTCA
GATCCTCCAGGAGACTTGTACACCATCTTTTGAGGCACAGAAACCCAATAGTCAACC
GCGGACTGGCATC (SEQ ID NO:113).

b) el promotor cysD de *Aspergillus nidulans*

AGATCTGGTTCCTGAGTACATCTACCGATGCGCCTCGATCCCCCTCTTAGCCGCATGA
GATTCCTACCATTTATGTCCTATCGTTCAGGGTCCTATTTGGACCGCTAGAAATAGAC
TCTGCTCGATTTGTTTCCATTATTCACGCAATTACGATAGTATTTGGCTCTTTTCGTTT
GGCCCAGGTCAATTCGGGTAAGACGCGATCACGCCATTGTGGCCGCCGGCGTTGTGC
TGCTGCTATTCCCCGCATATAAACAACCCCTCCACCAGTTCGTTGGGCTTTGCGAATG
CTGTACTCTATTTCAAGTTGTCAAAAGAGAGGATTAAAAAATTATACCCCAGATATC
AAAGATATCAAAGCCATC (SEQ ID NO:114).

c) un promotor cbh1 modificado que tiene la secuencia:

TCTAGAGTTGTGAAGTCGGTAATCCCGCTGTATAGTAATACGAGTCGCATCTAAATA
CTCCGAAGCTGCTGCGAACCCGGAGAATCGAGATGTGCTGGAAAGCTTCTAGCGAG
CGGCTAAATTAGCATGAAAGGCTATGAGAAATTCTGGAGACGGCTTGTTGAATCATG
GCGTTCCATTCTTCGACAAGCAAAGCGTTCCGTCGCAGTAGCAGGCACTCATTCCCG
AAAAAACTCGGAGATTCCTAAGTAGCGATGGAACCGGAATAATATAATAGGCAATA
CATTGAGTTGCCTCGACGGTTGCAATGCAGGGGTACTGAGCTTGGACATAACTGTTC
CGTACCCCACCTCTTCTCAACCTTTGGCGTTTCCCTGATTCAGCGTACCCGTACAAGT
CGTAATCACTATTAACCCAGACTGACCGGACGTGTTTTGCCCTTCATTTGGAGAAAA
TAATGTCATTGCGATGTGTAATTTGCCTGCTTGACCGACTGGGGCTGTTCGAAGCCCG
AATGTAGGATTGTTATTCCGAACTCTGCTCGTAGAGGCATGTTGTGAATCTGTGTCGG
GCAGGACACGCCTCGAAGGTTCACGGCAAGGGAAACCACCGATAGCAGTGTCTAGT

AGCAACCTGTAAAGCCGCAATGCAGCATCACTGGAAAATACAAACCAATGGCTAAA
AGTACATAAGTTAATGCCTAAAGAAGTCATATACCAGCGGCTAATAATTGTACAATC
AAGTGGCTAAACGTACCGTAATTTGCCAACGGCTTGTGGGGGTTGCAGAAGCAACG
GCAAAGCCCCACTTCCCCACGTTTGTTTCTTCACTCAGTCCAATCTCAGCTGGTGATC
CCCCAATTGGGTCGCTTGTTTGTTCCGGTGAAGTGAAAGAAGACAGAGGTAAGAATG
TCTGACTCGGAGCGTTTTGCATACAACCAAGGGCAGTGATGGAAGACAGTGAAATGT
TGACATTCAAGGGTATTTAGCCAGGGATGCTTGAGTGTATCGTGTAAGGAGGTTTGT
CTGCCGATACGACGAATACTGTATAGTCACTTCTGGTGAAGTGGTCCATATTGAAAT
GTAAGTCGGCACTGAACAGGAAAAGATTGAGTTGAAATGCCTAAGATCTCGGGCCC
TCGGGCCTTCGGGCCTTTGGGTGTACATGTTTGTGCTCCGGGCAAATGCAAAGTGTG
GTAGGATCGAACACACTGCTGCCTTTACCAAGCAGCTGAGGGTATGTGATAGGCAAA
TGTTCAGGGGCCACTGCATGGTTTCGAATAGAAAGAGAAGCTTAGCCTGCAGCCTCT
TATCGAGAAAGAAATTACCGTCGCTCGTGATTTGTTTGCAAAAAGAACAAAACTGAA
AAAACCAGACACGCTCGACTTCCTGTCTTCCTATTGATTGCAGCTTCCAATTTCGTCA
CACACAAGGTCCTAGCTTAGCCAAGAACAATAGCCGATAAAGATAGCCTCATTAAA
CGGAATGAGCTAGTAGGCAAAGTCAGCGAATGTGTATATATAAAGGTTCGAGGTCC
GTGCCTCCCTCATGCTCTCCCCATCTACTCATCAACTCAGATCCTCCAGGAGACTTGT
ACACCATCTTTTGAGGCACAGAAACCCAATAGTCAACCGCGGATGGCATC (SEQ ID
NO:115)

d) un promotor cysD de *Aspergillus nidulans* que tiene la secuencia:
AGATCTGGTTCCTGAGTACATCTACCGATGCGCCTCGATCCCCCTCTTAGCCGCATGA
GATTCCTACCATTTATGTCCTATTCGTTCAGGGTCCTATTTGGACCGCTAGAAATAGA
CTCTGCTTCGATTTGTTTCCATTATTCACGCAATTACGATAGTATTTGGCTCTTTTCGT
TTGGCCCAGGTCAATTCGGGTAAGACGCGATCACGCCATTGTGGCCGCCGGCGCCTG
CAGCCTCTTATCGAGAAAGAAATTACCGTCGCTCGTGATTTGTTTGCAAAAAGAACA
AAACTGAAAAACCAGACACGCTCGACTTCCTGTCTTCCTATTGATTGCAGCTTCCAAT
TTCGTCACACAACAAGGTCCTACGCCGGCGTTGTGCTGCTGCTATTCCCCGCATATAA
ACAACCCCTCCACCAGTTCGTTGGGCTTTGCGAATGCTGTACTCTATTTCAAGTTGTC
AAAAGAGAGGATTCAAAAAATTATACCCAGATATCAAAGATATCAAAGCCATC (SEQ
ID NO:116)

Se ha desarrollado una variedad de métodos para unir operablemente ADN a vectores a través de términos complementarios cohesivos. Por ejemplo, se pueden agregar tractos homopoliméricos complementarios al segmento de ADN a ser insertado en el vector de ADN. El vector y el segmento de ADN luego son unidos mediante unión de hidrógeno entre colas homopoliméricas complementarias para formar moleculas de ADN recombinante.

Los enlazadores sintéticos que contienen uno o más sitios de restricción proporcionan un método alternativo de unión de segmento de ADN a vectores. El segmento de ADN, generado mediante digestión de restricción de endonucleasa, es tratado con con ADN polimerasa del bateriófago T4 o ADN polimerasa I de E. Coli, enzimas las cuales remueven extremos sobresalientes de hebra sencilla gama, con sus actividad 3' 5' exonucleolíticas, y rellenan extremos 3' deprimidos con sus actividades polimerizantes.

La combinación de estas actividades por lo tanto genera segmentos de ADN de extremos burdos. Los segmentos de extremos burdos luego son incubados con un exceso molar grande de móleculas enlazadoras en presencia de una enzima que puede catalizar la ligación de moléculas de ADN de extremo burdo tal como ADN ligasa del bacteriofago T4. Por lo tanto, los productos de la reacción son segmentos de ADN que llevan secuencias enlazadoras poliméricas en sus extremos. Luego estos extremos de ADN son sometidos a escisión con la enzima de restricción

apropiada y ligados a un vector de expresión que ha sido hendido con una enzima que produce terminos compatibles con aquellos del segmento de ADN.

Los enlazadores sintéticos que contienen una variedad de sitios de endonucleasa de restricción son comercialmente disponibles a partir de un número de fuentes incluyendo International Biotechnologies Inc, New Haven, CT, USA.

Una forma deseable para modificar en ADN según el invento, si, por ejemplo se van a preparar variantes HA, es utilizar la reacción de cadena de polimerasa como se divulga en Saiki (1988) *Science* 239, 487-491. En este método el ADN a ser enzimáticamente amplificado es flanqueado por dos cebadores oligonucleótidos específicos los cuales se incorporan en el ADN amplificado. Los cebadores específicos pueden contener sitios de reconocimiento de endonucleasa de restricción los cuales se pueden utilizar para clonación en vectores de expresión utilizando métodos conocidos en el estado de la técnica.

Los generos de ejemplo de levadura contemplados en la práctica del presente invento como huespedes para expresión de proteínas de fusión de albúmina son *Pichia* (Hansenula), *Saccharomyces, Kluyveromyces, Candida, Torulopsis, Torulaspora, Schizosaccharomyces, citeromyces, Pachysolen, Debaromyces, Metschunikowia, Rhodosporidium, Leucosporidium, Botyyoascus, Sporidiobolus, Endomycopsis.* Generos preferidos son aquellos que constan de *Sacccharomyces, Schizosaccharomyces, Kluyveromyces, Pichia y Torulaspora.* Ejemplos de *Saccharomyces spp.* son S. *cerevisiae, S. italicus y S. rouxili..*

Ejemplos de Kluyveromyces spp. son K. fragilis K. lactis y K. marxianus. Una especie Torulaspora apropiada es T.delbrueckii. Ejemplos de Pichia (Hansenula) spp. son P. angusta (anteriormente H. polimorfa), P. anomala (anteriormente H. anormala) y P. pastoris. Métodos para la transformación de S.cerevisiae generalmente son ensañados en EP 251 744, EP 258 067 y WO 90/01063, todas incorporadas en la presente como referencia.

Especies de ejemplo preferidas de Saccharomyces incluyen S. cerevisiae, S. diastaticus, y Zygosaccharomyces rouxii. Especies de ejemplo preferidas de Saccharomyces incluyen S. cerevisiae, S. italicus, y Zygosaccharomyces rouxili. Especies de ejemplo preferidas de Kluyveromyces incluyen K- fragilis y K. lactis. Especies de ejemplo preferidas de Hansenula incluyen H. polymorfa (ahora Pichia angusta), H. anomala (ahora Pichia anomala), Pichia capsulata. Especies de ejemplo preferidas adicionales de Pichia incluyen P. pastoris. Especies de ejemplo preferidas de Aspergillus incluyen A. niger y A. nidulans. Especies de ejemplo preferidas de Yarrowia incluyen Y. lipolytica. Muchas especies de levadura preferidas son disponibles en ATCC. Por ejemplo, las siguientes especies de levadura preferidas están disponibles en ATCC y son útiles en la expresión de proteínas de fusión de albumina Sacccharomyces cerevisiae Hansen, cepa teleomófica BY4743 yap3 mutante (ATCC Accesión No. 4022731); Saccharomyces cerevisiae Hansen, Telemorph (ATCC Acceso Nos. 20626; 44774; y 62995); Saccharomyces diastaticus Andrews et Gilliland ex van der Walt, teleomorph (ATCC Accesion No. 62987); Kluyveromyces lactis (Dombrowski) van der Walt, teleomorph (ATCC Acceso No. 76492); Pichia angusta (Teunisson et al.) Kurtzman, teleomorfa depositada como Hansenula polimorfa de Morais et Maia, teleomorfa (ATCC Acceso No. 26012); Aspergillus niger van Tieghem, anamorfa (ATCC Accesion No. 16404); Aspergillus nidulans (Eidam) Winter, anamorfa (ATCC Acceso No. 48756); y Yarrowia lipolítica (wickerham et al.) van der Walt et von Arx, teleomorfa (ATCC Acceso No. 201847).

Promotores apropiados para *S. cerevisiae* incluyen aquellos asociados con el gen PGK1, genes GAL1 o GAL10, CYCI, PHO5, TRPI, ADHI, ADH2, los genes para glicerilaldehido-3-fosfato dehidrogenasa, hexoquinasa, pivurato decarboxilasa, fosfofructoquinasa, triosa fosfato isomerasa, fosfoglucosa isomerasa, glucoquinasa, feromona de factor de conjugación alfa, [una

feronoma de factor de conjugación], el promotor PRBI, el promotor GUT2, el promotor GPDI, y promotores híbridos que involucran híbridos de partes de regiones 5' reguladoras con partes de regiones 5' reguladoras de otros promotores o con sitios de activación corriente arriba (ej., el promotor de EP-A-258 067).

Promotores regulables convenientes para utilizar en *Schizosaccharomyces pombe* son el promotor tiamina-represible del gen nmt como se describe en Maundrell (1990) J. Biol. Chem 265, 10857-10864 y el gen promotor jbpl reprimible por glucosa como se describe en Hoffman & Winston (1990) *Genetics* 124, 807-816.

Métodos para transformar *Pichia* para expresión de genes extranjeros son enseñados en por ejemplo, Cregg (1993), y varias patentes de Phillips (ej., US 4 857 467, incorporada en la presente como referencia), y kits de expresión *Pichia* son comercialmente disponibles en Invitrogen BV, Leek, Netherlands, y Invitrogen Corp., San Diego, California. Promotores apropiados incluyen AOXI y AOX2. Gleeson (1986) J. Gen. Microbiol. 132, 3459-3465 incluye información sobre vectores Hansenula y transformación, promotores apropiados siendo MOX1 y FMD1; mientras EP 361 991, Fleer (1991) y otras publicaciones de Phone-Poulenc Rorer enseñan como expresar proteínas extranjeros en *Kluyveromyces* spp., un promotor apropiado es PGKI.

La señal de terminación de transcripción preferiblemente es la secuencia 3' flanqueadora de un gen eucariótico el cual contiene señales adecuadas para terminación de transcripción y polialdenilación. Las secuencias 3' flanqueadoras adecuadas por ejemplo, pueden ser aquellas del gen naturalmente ligado a la secuencia de control de expresión utilizada, es decir, puede corresponder al promotor. Alternativamente, estas pueden ser diferentes en cuyo caso la señal de terminación del gen ADHI de *s. cervisiae* es preferido.

La proteína de fusión de albúmina deseada inicialmente se puede expresar con una secuencia guía de secreción, la cual puede ser cualquier secuencia guía efectiva en la levadura elegida. Secuencias guía útiles en levadura incluyen cualquiera de las siguientes:

a) la secuencia de señal MPIF (ej., aminoácidos 1-21 GenBank número de acceso AAB51134) MKVSVAALSCLMLVTALGSQA (SEQ ID NO:6)
b) la secuencia de señal de estaniocalcina (MLQNSAVLLLVISASA, SEQ ID NO:7)
c) la región pre-pro de la secuencia de señal HSA (ej., MKWVTFISLLFLFSSAYSRGVFRR; SEQ ID NO:8)
d) la pre región de la secuencia de señal HSA (ej., MKWVTFISLLFLFSSAYS, SEQ ID:NO:9) o variantes de la misma, tal como, por ejemplo, MKWVSFISLLFLFSSAYS, (SEQ ID NO:10)
e) la secuencia de señal de invertasa (ej., MLLQAFLFLLAGFAAKISA, SEQ ID NO:11)
f) la secuencia de señal de factor de conjugación alfa de levadura (ej., MRFPSIFTAVLAFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPESNSTN NGLLFINTTIASSIAAKEEGVSLDKR, SEQ, ID NO: 12).

g) secuencia guía de toxinas asesinas *K. lactis*
h) una secuencia de señal híbrida (ej., MKWVSFISLLFLFSSAYSRSLEKR, SEQ ID NO:13)
i) una secuencia de señal hibridas HSA/MFα-1 (también conocida como HSA/kex2)(ej., MKWVSFISLLFLFSSAYSRSLDKR, SEQ ID NO:14)
j) una secuencia guía de fusión asesina *K.lactis*/MFα-1 (ej., MNIFYFLFLLSFVQGSLDKR, SEQ ID NO:15)
k) la secuencia de señal de inmunoglobulina Ig (ej., MGWSCIILFLVATATGVHS, SEQ ID

NO:16)
l) la secuencia de señal precursoras de Fibulina B (ej., MERAAPSRRVPLPLLLLGGLALLAAGVDA, SEQ ID NO:17)
m) la secuencia de señal precursoras de clusterina (ej., MMKTLLLFVGLLLTWESGQVLG, SEQ ID NO:18)
n) la secuencia de señal de proteína 4 fijadora de factor de crecimiento tipo insulina (ej., MLPLCLVAALLLAAGPGPSLG, SEQ ID NO:19)
o) variantes de la pre-pro-region de la secuencia de señal HSA tal como, por ejemplo, MKWVSFISLLFLFSSAYSRGVFRR (SEQ. ID NO:20)
MKWVTFISLLFLFAGVLG (SEQ. ID NO:21),
MKWVTFISLLFLFSGVLG (SEQ. ID NO:22),
MKWVTFISLLFLFGGVLG (SEQ. ID NO:23),
HSA guía de HSA modificada # 64-MKWVTFISLLFLFAGVSG (SEQ. ID NO:24),
HSA guía de HSA modificada #66-MKWVTFISLLFLFGGVSG (SEQ. ID NO:25),
Guía de HSA modificada (A14) -MKWVTFISLLFLFAGVSG (SEQ. ID NO:26);
Guía de HSA modificada (S14) (también conocida como HSA #65 modificada - MKWVTFISLLFLFSGVSG (SEQ. ID NO:27);
Guía HSA modificada (G14)-MKWVTFISLLFLFGGVSG (SEQ. ID NO:28), o MKWVTFISLLFLFGGVLGDLHKS (SEQ. ID NO:29)
p) una secuencia de señal de consenso (MPTWAWWLFLVLLLALWAPARG, SEQ ID NO:30)
q) secuencia guía de fosfatasa ácida (PH05) (ej., MFKSVVYSILAASLANA SEQ ID NO:31)
r) la pre-secuencia de MFoz-1
s) la pre-secuencia de glucanasa 0 (BGL2)
t) secuencia guía de toxina asesina
u) la presecuencia de toxina asesina
v) toxina asesina *k. lactis* prepro (29 aminoácidos; 16 aminoácidos de pre y 13 aminoácidos de pro)
w) secuencia guía de secreción de glucoamilasa II del *S.diastaticus*
x) secuencia guía de secreción de α-galactosidasa (MELI) del *S.carsbergensis* (MELI)
y) secuencia guía de *candida glucoarnylasa*
z) Las secuencias guia híbridas divulgadas en EP-A-387 319 (incorporadas en la presente como referencia)
aa) la secuencia de señal gp67 (en conjunción con sistemas de expresión baculoviral) (ej., aminoácidos 1-19 de GenBank Número de Acceso AAA72759) o
bb) la secuencia guía natural de la proteína terapéutica X;
cc) secuencia guía de S. cerevisiae (SUC2), como se divulgo en JP 62-096086 (otorgada como 911036516, incorporada en la presente como referencia); o
dd) Inulinasa – MKLAYSLLLPLAGVSASVINYKR (SEQ ID NO:33).
ee) Un variante #1 de la secuencia guía del propéptido TA57 modificado - MKLKTVRSAVLSSLFASQVLGQPIDDTESQTTSVNLMADDTESAFATQTNSGGLDVVGL ISMAKR (SEQ ID NO:34)
ff) Un variante #2 de la secuencia guía del propéptido TA57 modificado – MKLKTVRSAVLSSLFASQVLGQPIDDTESQTTSVNLMADDTESAFATQNSGGLDVVGLIS MAEEGEPKR (SEQ ID NO:35)
gg) Un péptido de señal de consenso – MWWRLWWLLLLLLLLWPMVWA (SEQ ID NO:111)
hh) Una secuencia de señal HSA/kex2 modificada- MKWVSFISLLFLFSSAYSGSLDKR (SEQ ID NO:112)

ii) Un péptido de señal de consenso #2 – MRPTWAWWLFLVLLALWAPARG (SEQ ID NO:105)

Métodos Adicionales de Producción Recombinante y Sintética de Proteínas de Fusión de Albúmina

El presente invento también se relaciona con vectores que contienen un polinucleotido que codifica una proteínas fusión de albúmina del presente invento, células huesped, y la producción de proteínas de fusión de albúmina mediante técnicas sintéticas y recombinantes. El vector puede ser, por ejemplo, un fago, plásmido, vector viral, o retroviral. Los vectores retrovirales pueden ser de replicación competente o de replicación defectuosa. En el último caso la propagación viral generalmente ocurrirá sólo en células huesped complementarias.

Los polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden estar unidos a un vector que contiene un marcador para propagación en un huesped. Generalmente, un vector plásmido es introducido en un precipitado, tal como un precipitado de fosfato de cálcio, o en un complejo con un lípido cargado. Si el vector es un virus, éste se puede empacar in vitro utilizando una línea celular empacada apropiada y luego transducida a células huesped.

El inserto nucleotido debe estar operativamente ligado a un promotor apropiado, tal como el promotor lambda PL fago, los promotores de *E. coli lac, trp. phoA y tac*, los promotores temprano y tardio SV40 y promotores de LTRs retroviral, por nombrar solo algunos. Otros promotores apropiados serán conocidos por alguien con experiencia en el estado de la técnica. Los constructos de expresión contendrán adicionalmente sitios para iniciación, terminación de transcripción, y en la región transcrita, un sitio de unión de ribosomas para traslación. La porción codificadora de los transcriptos expresada por los constructos preferiblemente incluirá un codon iniciador de traslación al comienzo y un codon de terminación (UAA, UGA) apropiadamente colocado al final del polipéptido a ser trasladado.

Como se indica, los vectores de expresión preferiblemente incluirán un marcador seleccionable. Tales marcadores incluyen reductasa de hidrofolato, G418, sintasa de glutamina, o resistencia de neomicina para cultivo de células eucarióticas, y genes de resistencia a tetraciclina, canamicina, o ampicilina para cultivar *E. coli* y otras bacterias. Ejemplos representativos de huespedes apropiados incluyen, pero no están limitados a, células bacterianas, tales como células de *E. coli*, Streptomyces y *salmonella typhimurium;* células micóticas, tales como células de levadura (ej., *Saccharomyces cerevisiae* o *Pichia postoris* (Número de Acceso ATCC No. 201178)); células de insectos tales como Drosophilia S2 y Spodoptera Sf9; células animales tales como CHO, COS, NSO, 293, y células de melanona Bowes; y células vegetales. Medios y condiciones de cultivo apropiados para las células huesped anteriormente descritas son conocidos en el estado de la técnica.

Entre los vectores preferidos para utilizar en bacterias se incluyen pQE70, pQE60 y pQE-9, disponibles en QIAGEN, Inc.; vectores pBluescript, vectores Phagescript, pNH8A, pNH16a, pNH18A, pNH46A, disponibles en Stratagene Cloning Systems, Inc.; y ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 disponibles en Pharmacia Biotech, Inc. Entre los vectores eucarióticos preferidos están pWLNEO, pSV2CAT, pOG44, pXTI y pSG disponibles en Stratagene; y pSVK3, pBPV, pMSG y pSVL disponibles en Pharmacia. Vectores de expresión preferidos para utilizar en sistema de levadura incluyen, pero no están limitados a pYES2, pYD1, pTEFI/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, y PAO815 (todos disponibles en Invitrogen, Carlbad, CA). Otros vectores apropiados serán facilmente aparentes para alguien con experiencia en el estado de la técnica.

En una realización, los polinucleotidos que codifican una proteína de fusión de albúmina del invento se pueden fusionar a secuencias de señales las cuales dirigirán la localización de una proteína del invento a compartimientos particulares de una célula procariótica o eucariótica y/o dirigirán la secreción de una proteína del invento desde una célula procariotica o eucariotica. Por ejemplo, en *E. coli*, puede ser deseable dirigir la expresión de la proteína al espacio periplásmico. Ejemplos de proteínas o secuencias de señales (o fragmentos de las mismas) a las cuales las proteínas de fusión de albúmina del invento se pueden fusionar para dirigir la expresión del polipéptido al espacio periplásmico de bacterias incluyen, pero no están limitadas a la secuencia de señal *pelB*, la secuencia de señal de proteína de unión de maltosa (MBP), MBP, la secuencia de señal *ompA*, la secuencia de señal de la subunidad B periplásmica de la enterotoxina termolábil de E. coli, y la secuencia de señal de fosfatasa alcalina. Varios vectores son comercialmente disponibles para la construcción de proteínas de fusión los cuales dirigirán la localización de una proteína, tal como la serie pMAL de vectores (particularmente la serie pMAL-p) disponible en New England Biolabs. En una realización específica, polinucleótidos de proteínas de fusión de albúmina del invento se pueden fusionar a la secuencia de señal de lyasa de peptato *pelB* para aumentar la eficiencia de expresión y purificación de tales polipéptidos en bacterias *Gram*-negativas. Ver las Patentes Americanas No. 5.576.195 y 5.846.818, incorporadas en la presente como referencia en su totalidad.

Ejemplos de péptidos de señal que se pueden fusionar a una proteína de fusión de albúmina del invento para dirigir su secreción en células mamíferas incluyen, pero no están limitados a:

a) la secuencia de señal MPIF (ej., aminoácidos 1-21 GenBank número de acceso AAB51134) MKVSVAALSCLMLVTALGSQA (SEQ ID NO:6)
b) la secuencia de señal de estaniocalcina (MLQNSAVLLLVISASA, SEQ ID NO:7)
c) la región pre-pro de la secuencia de señal HSA (ej., MKWVTFISLLFLFSSAYSRGVFRR; SEQ ID NO:8)
d) la pre región de la secuencia de señal HSA (ej., MKWVTFISLLFLFSSAYS, SEQ ID:NO:9) o variantes de la misma, tal como, por ejemplo, MKWVSFISLLFLFSSAYS, (SEQ ID NO:10)
e) la secuencia de señal de invertasa (ej., MLLQAFLFLLAGFAAKISA, SEQ ID NO:11)
f) la secuencia de señal de factor de conjugación alfa de levadura (ej., MRFPSIFTAVLAFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPESNSTN NGLLFINTTIASSIAAKEEGVSLDKR, SEQ, ID NO: 12).

g) secuencia guía de toxinas asesinas *K. lactis*
h) una secuencia de señal híbrida (ej., MKWVSFISLLFLFSSAYSRSLEKR, SEQ ID NO:13)
i) una secuencia de señal hibridas HSA/MFα-1 (también conocida como HSA/kex2)(ej., MKWVSFISLLFLFSSAYSRSLDKR, SEQ ID NO:14)
j) una secuencia guía de fusión asesina *K.lactis*/MFα-1 (ej., MNIFYFLFLLSFVQGSLDKR, SEQ ID NO:15)
k) la secuencia de señal de inmunoglobulina Ig (ej., MGWSCIILFLVATATGVHS, SEQ ID NO:16)
l) la secuencia de señal precursoras de Fibulina B (ej., MERAAPSRRVPLPLLLLGGLALLAAGVDA, SEQ ID NO:17)
m) la secuencia de señal precursoras de clusterina (ej., MMKTLLLFVGLLLTWESGQVLG, SEQ ID NO:18)

n) la secuencia de señal de proteína 4 fijadora de factor de crecimiento tipo insulina (ej., MLPLCLVAALLLAAGPGPSLG, SEQ ID NO:19)
o) variantes de la pre-pro-region de la secuencia de señal HSA tal como, por ejemplo, MKWVSFISLLFLFSSAYSRGVFRR (SEQ. ID NO:20)
MKWVTFISLLFLFAGVLG (SEQ. ID NO:21),
MKWVTFISLLFLFSGVLG (SEQ. ID NO:22),
MKWVTFISLLFLFGGVLG (SEQ. ID NO:23),
HSA guía de HSA modificada # 64-MKWVTFISLLFLFAGVSG (SEQ. ID NO:24),
HSA guía de HSA modificada #66-MKWVTFISLLFLFGGVSG (SEQ. ID NO:25),
Guía de HSA modificada (A14) -MKWVTFISLLFLFAGVSG (SEQ. ID NO:26);
Guía de HSA modificada (S14) (también conocida como HSA #65 modificada - MKWVTFISLLFLFSGVSG (SEQ. ID NO:27);
Guía HSA modificada (G14)-MKWVTFISLLFLFGGVSG (SEQ. ID NO:28), o MKWVTFISLLFLFGGVLGDLHKS (SEQ. ID NO:29)
p) una secuencia de señal de consenso (MPTWAWWLFLVLLLALWAPARG, SEQ ID NO:30)
q) secuencia guía de fosfatasa ácida (PH05) (ej., MFKSVVYSILAASLANA SEQ ID NO:31)
r) la pre-secuencia de MFoz-1
s) la pre-secuencia de glucanasa 0 (BGL2)
t) secuencia guía de toxina asesina
u) la presecuencia de toxina asesina
v) toxina asesina *k. lactis* prepro (29 aminoácidos; 16 aminoácidos de pre y 13 aminoácidos de pro)
w) secuencia guía de secreción de glucoamilasa II del *S.diastaticus*
x) secuencia guía de secreción de α-galactosidasa (MELI) del *S.carsbergensis* (MELI)
y) secuencia guía de *candida glucoarnylasa*
z) Las secuencias guia híbridas divulgadas en EP-A-387 319 (incorporadas en la presente como referencia)
aa) la secuencia de señal gp67 (en conjunción con sistemas de expresión baculoviral) (ej., aminoácidos 1-19 de GenBank Número de Acceso AAA72759) o
bb) la secuencia guía natural de la proteína terapéutica X;
cc) secuencia guía de S. cerevisiae (SUC2), como se divulgo en JP 62-096086 (otorgada como 911036516, incorporada en la presente como referencia); o
dd) Inulinasa – MKLAYSLLLPLAGVSASVINYKR (SEQ ID NO:33).
ee) Un variante #1 de la secuencia guía del propéptido TA57 modificado - MKLKTVRSAVLSSLFASQVLGQPIDDTESQTTSVNLMADDTESAFATQTNSGGLDVVGL ISMAKR (SEQ ID NO:34)
ff) Un variante #2 de la secuencia guía del propéptido TA57 modificado – MKLKTVRSAVLSSLFASQVLGQPIDDTESQTTSVNLMADDTESAFATQNSGGLDVVGLIS MAEEGEPKR (SEQ ID NO:35)
gg) Un péptido de señal de consenso – MWWRLWWLLLLLLLLWPMVWA (SEQ ID NO:111)
hh) Una secuencia de señal HSA/kex2 modificada- MKWVSFISLLFLFSSAYSGSLDKR (SEQ ID NO:112)
ii) Un péptido de señal de consenso #2 – MRPTWAWWLFLVLLALWAPARG (SEQ ID NO:105)

En una realización preferida, la secuencia de señal HSA/kex2 modificada (SEQ ID NO:112) está fusionada al extremo amino de una proteína de fusión de albúmina, incluyendo proteínas de fusión que comprenden albúmina y una proteína terapéutica como se describe en la

presente, así como también proteínas de fusión de albúmina divulgadas en WO93/151199; WO97/24445; WO03/60071; WO03/59934; y PCT/US04/01369, las cuales son incorporadas en la presente como referencia. La secuencia de señal HSA/kex2 se basa en la secuencia de señal HSA/kex2 (SEQ ID NO:14) divulgada, ej., en Sleep, Biotechnology 1990, vol 8, pp. 42-46; y Patente Americana 5.302.697, ambas incorporadas en la presente como referencia en su totalidad. La secuencia guía HSA/kex2 divulgada en la presente contiene una sustitución aminoácido no conservadora (Arg a Gly) en el residuo 19 del péptido de señal precursor. Se ha encontrado que el péptido de señal precursor HSA/kex2 produce rendimiento de expresión inesperadamente mejor y/o mejor eficiencia de escisión de proteínas de fusión de albúmina cuando se expresa en levadura que la secuencia de señal HSA/kex2 no modificada. Las variantes del polipéptido de señal HSA/kex2 modificado son contempladas por el invento. En particular el residuo Gly en la posición de SEQ ID NO:112 se puede sustituir con Pro. También se contemplan otras variantes de sustitución conservadoras de la secuencia de señal HSA/kex2 modificada. Los ácidos nucleicos que codifican la secuencia de señal HSA/kex2 modificada de SEQ ID NO:112, así como también variantes de sustitución conservadora de las mismas, son contemplados por el invento.

Los vectores que utilizan glutamina sintasa (GS) o DHFR como marcadores seleccionables se pueden amplificar en la presencia de los fármacos metionina sulfoximina o metotrexato, respectivamente. Una ventaja de vectores basados en glutamina sintasa es la disponibilidad de líneas celulares (ej., la línea celular de mieloma murino, NSO) las cuales son glutamina sintasa negativas. Los sistemas de expresión de glutamina sintasa también pueden funcionar en células de expresión de glutamina sintasa (ej., células de Ovario de Hamster Chino (CHO) al proporcionar inhibidor adicional para prevenir el funcionamiento del gen endógeno. Un sistema de expresión de glutamna sintasa y componentes del mismo son detallados en las publicaciones PCT: WO87/04462; WO86/05807; WO89/01036; WO89/10404; y WO01/06657, las cuales se incorporan en el presente como referencia. Adicionalmente, los vectores de expresión de glutamina sintasa se pueden obtener en Lonza Biologics, Inc. (Portsmouth, NH). La expresión y producción de anticuerpos monoclonales utilizando un sistema de expresión GS en células de mieloma murino se describe en Bebbington, *Bio/tecnology* 10:169(1992) y en Biblia y Robinsson *Biotechnol. Prog* 11:1 (1995) los cuales se incorporan en la presente como referencia

El presente invento también se relaciona con células huésped que contienen los constructor de vectores anteriormente descritos en la presente, y adicionalmente contempla células huésped que contienen secuencias nucleótidas del invento que son operablemente asociadas con una o más regiones de control heterólogas (ej., promotor y/o mejorador) utilizando técnicas conocidas en el estado de la técnica. La célula hueste puede ser una célula eucariotica superior, tal como una célula mamífera (ej., una célula derivada humana) o una célula eucariotica inferior, tal como una célula de levadura, o la célula huésped puede ser una célula procariótica, tal como una célula bacteriana. Se puede elegir una célula huésped que module la expresión de las secuencias genéticas insertadas, o modifique y procese el producto genético en la forma específica deseada. La expresión de ciertos promotores se puede elevar en presencia de ciertos inductores; por lo tanto la expresión del polipéptido geneticamente diseñado se puede controlar. Además, diferentes células huésped tienen caracterísitas y mecanismos específicos para modificación y procesamiento traslacional y post-traslacional (ej., forforilación, división) de proteínas. Las líneas celulares apropiadas se pueden elegir para asegurar las modificaciones deseadas y procesamiento de la proteína extraña expresada.

La introducción de los ácidos nucleicos y constructos de ácidos nucleicos del invento en la célula huésped se puede realizar mediante transfección de fosfato de calcio, transfección

mediada por DEAE-dextrana, transfección mediada por lípidos catiónicos, electroporación, transducción, infección, u otros métodos. Tales métodos se describen en muchos manuales de laboratorio estándares, tales como Davis, Basic Methods in Molecular Biology (1986). Específicamente se contempla que los polipéptidos del presente invento se pueden en realidad expresar mediante una célula huésped que carece de un vector recombinante. Además de contemplar células huésped que contienen los constructos de vector discutidos en la presente, el invento también contempla células huésped primarias, secundarias, e inmortalizadas de origen vertebrado, particularmente origen mamífero, que han sido modificadas para suprimir o reemplazar material genético endógeno (ej., la secuencia codificadora correspondiente a una proteína terapéutica se puede reemplazar con una proteína de fusión de albúmina correspondiente a la proteína Terapéutica) y/o para incluir material genético (ej., secuencias polinucleotidas heterólogas tales como por ejemplo, una proteína de fusión de albúmina del invento correspondiente a la proteína Terapéutica se puede incluir). El material genético asociado de manera operable con el Polinucleótido endógeno puede activar, y/o amplificar polinucleótidos endógenos.

Además, las técnicas conocidas en el estado de la técnica se pueden utilizar para asociar de manera operable polinucleótidos heterólogos (ej., polinucleótidos que codifican un proteína de albúmina, o un fragmento o variante de la misma) y/o regiones de control heterólogo (ej., promotor y/o mejorador) con secuencias polinucleotidas endógenas que codifican un proteína Terapéutica a través de recombinación homóloga (ver, ej., Patente Americana No. 5.641.670, publicada en Junio 24 de 1997; Número de Publicación Internacional WO 96/29411; Número de Publicación Internacional WO 94/12650; Koller, *Proc. Natl. Acad. Sci. USA 86:*8932-8935 (1989); y Zijlstra *Nature* 342:435-438 (1989), las divulgaciones de estas se incorporan en la presente como referencia).

Las proteínas de fusión de albúmina del invento se pueden recuperar y purificar a partir de cultivos de células recombinantes mediante métodos bien conocidos incluyendo sulfato de amonio o precipitación de etanol, extracción de ácido, cromatografía de intercambio aniónico o catiónico, cromatografía de fosfocelulosa, cromatografía de interacción hidrofóbica, cromatografía de afinidad, cromatografía de hidroxilapatita, cromatografía de interacción de carga hidrofóbica y cromatografía de lectina. Más preferiblemente, se utiliza cromatografía liquida de alto desempeño ("HPLC" para purificación.

En realizaciones preferidas las proteínas de fusión de albúmina son purificadas utilizando Cromatografía de Intercambio Aniónico incluyendo, pero sin limitar a. cromatografía sobre Q-sefarosa, DEAE sefarosa, poros HQ, poros DEAE, Toyopearl Q, Toyopearl QAE, Toyopearl DEAE, Resource/Source Q y DEAD, Fractogel Q y columnas DEAE.

En realizaciones específicas las proteínas de fusión de albúmina del invento son purificadas utilizando Cromatografía de Intercambio Cationico incluyendo, pero sin limitar a SP-sefarosa, CM sefarosa, poros HS, poros CM, Toyopearl CM, Resourse/Source S y CM, columnas de Fractogel S y CM y sus equivalentes y comparables.

En realizaciones específicas las proteínas de fusión de albúmina del invento son purificadas utilizando Cromatografía de Intercambio Cationico incluyendo, pero sin limitar a, SP-sefarosa, CM sefarosa, poros HS, poros CM, Toyopearl SP, Toyopearl CM, Resourse/Source S y CM, columnas de Fractogel S y CM y sus equivalentes y comparables.

En realizaciones específicas las proteínas de fusión de albúmina del invento son purificadas utilizando Cromatografía de Interacción Hidrofóbica incluyendo, pero sin limitar a columnas de Fenilo, Butilo, Metilo, Octilo, Hexil-Sefarosa, poros Fenilo, Butilo, Metilo, Octilo, Hexilo, Toyopearl Fenilo, Butilo, Metilo, Octilo, Hexilo, Resource/Source Fenilo, Butilo, Metilo,

Octilo, Hexilo, Fractogel Fenilo, Butilo, Metilo, Octilo, Hexilo y sus equivalentes y comparables.

En realizaciones específicas las proteínas de fusión de albúmina del invento son purificadas utilizando Cromatografía de Exclusión de Tamaño incluyendo, pero sin limitar a sefarosa S100, S200, S300, columnas de resina superdex y sus equivalentes y comparables.

En realizaciones específicas las proteínas de fusión de albúmina del invento son purificadas utilizando Cromatografía de Afinidad incluyendo, pero sin limitar a columnas de afinidad de tintes miméticos, afinidad de péptidos y afinidad de anticuerpos que son selectivas para la HSA o las moléculas "objetivo de fusión".

En realizaciones preferidas las proteínas de fusión de albúmina del invento son purificadas utilizando uno o más métodos de Cromatografía listados anteriormente. En otras realizaciones preferidas, las proteínas de fusión de albúmina del invento son purificadas utilizando una o más de las siguientes columnas de Cromatografía, columna de Q Sefarosa FF, columna de SP Sefarosa FF, columna de Alto Desempeño de Sefarosa Q, columna Blue Sepharosa FF, Columna Blue, Columna de Fenilo Sefarosa FF, Sefarosa DEAE FF, o columna de Metilo.

Adicionalmente, las proteínas de fusión de albúmina del invento se pueden purificar utilizando el proceso descrito en la Publicación Internacional PCT WO 00/44772 la cual es incorporada en la presente como referencia en su totalidad. Alguien con experiencia en el estado de la técnica fácilmente podría modificar el proceso descrito en la presente para utilizar en purificación de proteínas de fusión de albúmina del invento.

Las proteínas de fusión de albúmina del presente invento se pueden recuperar a partir de productos de procedimientos sintéticos químicos; y productos producidos mediante técnicas recombinantes a partir de un huésped procariótico o eucariótico, incluyendo por ejemplo, células bacterianas, de levadura, vegetales superiores, de insectos, y células mamíferas. Dependiendo del huésped empleado en una procedimiento de producción recombinante, los polipéptidos del presente invento pueden ser glicosilados o pueden ser no glicosilados. Además, las proteínas de fusión de albúmina del invento también pueden incluir un residuo de metionina modificado inicial, en algunos casos como resultado de procesos mediados por huéspedes. Por lo tanto, es bien sabido en el estado de la técnica que la metionina N-terminal codificada por el codon de iniciación de traslación generalmente es removida con alta eficiencia a partir de cualquier proteína después de traslación en todas las células eucarioticas. Mientras la metionina N-terminal sobre la mayoría de proteínas también es retirada de manera eficiente en la mayoría de procariotos, para algunas proteínas, este proceso de remoción procariótico es ineficiente, dependiendo de la naturaleza del aminoácido al cual la metionina N-terminal está covalentemente ligada.

En una realización, la levadura *Pichia pastoris* se utiliza para expresar proteínas de fusión de albúmina del invento en un sistema eucariótico. *Pichia pastoris* es una levadura metilotrófica la cual puede metabolizar metanol como su únic fuente de carbono. Un paso principal en la ruta de metabilización de metanol es la oxidación de metanol a formaldehído utilizando O2. Esta reacción es catalizada por la enzima alcohol oxidasa. Con el objeto de metabolizar metanol como su única fuente de carbono, *Pichia pastoris* debe generar altos niveles de alcohol oxidasa debido en parte a la afinidad relativamente baja de la alcohol oxidasa para O2. Consecuentemente, en un medio de crecimiento dependiendo del metanol como una fuente principal de carbono, la región promotora de uno de los dos genes de alcohol oxidasa (AOXI) es altamente activa. En presencia de metanol, la alcohol oxidasa producida a partir del gen AOXI comprende hasta aproximadamente 30% de la proteína soluble total en *Pichia pastoris*. Ver Ellis, S.B. *Mol. Cell Biol.* 5:1111-21 (1985); Koutz, P.J. *Yeast* 5:167-77 (1989); Tschopp, J.F., *Nucl. Acids Res.*

15:3859-76 (1987). Por lo tanto, una secuencia codificadora heteróloga, tal como, por ejemplo, un polipéptido del presente invento, bajo la regulación transcripcional de toda o parte de la secuencia reguladora AOXI, se expresa a niveles excepcionalmente altos en levadura *Pichia* cultivada en presencia de metanol.

En un ejemplo, el vector plásmido pPIC9K se utiliza para expresar ADN que codifica una proteína de fusión de albúmina del invento, como se divulga en la presente, en un sistema de levadura *pichia* esencialmente como se describe en "*Pichia Protocols*: Methods in Molecular Biology, " D.R. Higgins y J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. Este vector de expresión permite la expresión y secreción de un polipéptido del invento en virtud del promotor AOXI fuerte ligado al péptido de señal secretoria de fosfatasa alcalina *Pichia Pastoris* (es decir, guía) localizado corriente arriba de un sitio de clonación múltiple.

Muchos otros vectores de levadura se pueden utilizar en cambio de pPIC9K, tal como, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalfa, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, y PAO815, como alguien con experiencia en el estado de la técnica fácilmente apreciaría, siempre y cuando el constructo de expresión propuesto suministre señales apropiadamente localizadas para transcripción, traslación, secreción (si se desea), y similares, incluyendo un AUG en marco de lectura correcto tal como se requiera.

En otra realización, la expresión de alto nivel de una secuencia codificadora heteróloga, tal como, por ejemplo, un Polinucleótido que codifica una proteína de fusión de albúmina del presente invento, se puede lograr mediante clonación del polipéptido heterólogo del invento en un vector de expresión tal como, por ejemplo, pGAPZ o pGAPZalfa, y desarrollando el cultivo de levadura en ausencia de metanol.

Además, las proteínas de fusión de albúmina del invento se pueden sintetizar de manera química utilizando técnicas conocidas en el estado de la técnica (ej., ver Creigton, 1983, Proteínas: Estructuras y Principios Moleculares, W.H. Freeman & Co. N.Y. y Hunkapiller, *Nature,* 310:105-111 (1984)). Por ejemplo, un polipéptido que corresponde a un fragmento de un polipéptido se puede sintetizar mediante el uso de un sintetizador de péptidos. Además, si se desea, aminoácidos no clásicos o análogos de aminoácidos químicos se pueden introducir como una sustitución o adición en la secuencia polipeptida. Los aminoácidos no clasicos incluyen, pero no están limitados a los isómeros S de los aminoácidos comunes, ácido 2,4-diamino-butírico, ácido amino-isobutírico, ácido 4-amino-butírico, Abu, ácido 2-amino-butírico, g-Abu, e-Ahx, ácido 6-amino hexánoico, Aib, ácido 2 amino isobutírico, ácido 3-amino propiónico, ornitina, norleucina, norvalina, hidroxiprolina, sarcosina, citrulina, homocitrulina, ácido cisteico, t-butilglicina, t-butilalanina, fenilglicina, ciclohexilalanina, b-alanina, ácidos fluoroamino, aminoácidos de diseño tales como aminoácidos de b-metilo, aminoácidos de Ca-metilamino, aminoácidos de Ca-metil- amino, aminoácidos de Na-metilo, y análogos de aminoácidos en general. Además, el aminoácido puede ser D (dextrorotatorio) o L (levorotatorio).

El invento contempla proteínas de fusión de albúmina del presente invento las cuales son diferencialmente modificadas durante o después de traslación, ej., mediante glicosilación, acetilación, fosforilación, amidación, derivatización mediante grupos protectores/bloqueadores conocidos, división proteolítica, enlace a una molécula de anticuerpo u otro ligando celular, etc. Cualquiera de las modificaciones químicas se puede realizar mediante técnicas conocidas, incluyendo pero sin limitar a división química específica mediante bromuro de cianogeno, tripsina, quimotripsina, papaina, proteasa V8, NaBH4, acetilación, formilación, oxidación, reducción; síntesis metabólica en presencia de tunicamicina; etc.

Modificaciones post-traslacionales adicionales contempladas por el invento incluyen, por ejemplo, cadenas de carbohidra N-ligadas o O-ligadas, procesamiento de extremos N-terminales

o C-terminales, fijación de fracción químicas al esqueleto del aminoácido, modificaciones químicas de cadenas de carbohidrato N-ligadas o O-ligadas, y adición o eliminación de un residuo de metionina N-terminal como resultado de expresión celular de huésped procariótico. Las proteínas de fusión de albúmina también se pueden modificar con un rotulo detectable, tal como un rotulo enzimático, fluorescente, isotópico o de afinidad para permitir la detección y aislamiento de la proteína.

Ejemplos de enzimas apropiadas incluyen peroxidasa de rábano, fosfatasa alcalina, beta-galactosidasa, o acetilcolinesterasa; ejemplos de complejos de grupos prostéticos adecuados incluyen estreptavidina/biotina u avidin/biotina; ejemplos de materiales fluorescentes apropiados incluyen umbeliferota, floresceina, isotiocianato de fluoresceína, rodamina, fluoresceína de diclorotriazinilamina, cloruro de dansilo, o ficoeritrina; un ejemplo de un material luminiscente incluye luminol; ejemplos de materiales bioluminiscentes incluyen luciferasa, luciferina, y aequorina; y ejemplos de material radioactivo apropiado incluyen yodo (^{121}I, ^{123}I, ^{125}I, ^{131}I), carbono (^{14}C), azufre (^{35}S), tritio (^{3}H), indio (^{111}In, ^{112}In, ^{113m}In, ^{115m}In), tecnetio (^{99}Tc, ^{99m}Tc), talio (^{201}Ti), galio (^{68}Ga, ^{67}Ga), paladio(^{103}Pd), molibdeno (^{99}Mo), xenon (^{133}Xe), fluoro(^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, y ^{97}Ru.

En realizaciones específicas, las proteínas de fusión de albúmina del presente invento o fragmentos o variantes de las mismas están unidas a quelantes macrocíclicos que se asocian con iones radiometálicos, incluyendo pero sin limitar a ^{177}Lu, ^{90}Y, ^{166}Ho, y ^{153}Sm a polipéptidos. En una realización preferida, el ión radiometálico asociado con los quelantes macrocíclicos es ^{111}In.En otra realización preferida, el ion radiometálico asociada con el quelante macrocíclico es ^{90}Y. En realizaciones específicas, el quelante macrolítico es ácido 1,3,7,10-tetraazaciclododecano-N,N',N'',N'''-tetraacético (DOTA). En otras realizaciones específicas, DOTA está unido a un anticuerpo del invento o fragmento del mismo a través de una molécula enlazadora. Ejemplos de moléculas enlazadoras útiles para conjugar DOTA a un polipéptido son comúnmente conocidos en el estado de la técnica – ver por ejemplo, DeNardo, Clin Cáncer Res. 4(10):2483-90 (1998); Peterson, Bioconjug. Chem. 10(4):553-7 (1999); y Zimmerman, Nucl. Med. Biol. 26(8):943-50 (1999); las cuales están incorporadas en la presente como referencia en su totalidad.

Como se mencionó, las proteínas de fusión de albúmina del invento se pueden modificar ya sea mediante procesos naturales, tal como procesamiento post-traslacional, o mediante técnicas de modificación química las cuales son bien conocidas en el estado de la técnica. Se apreciará que el mismo tipo de modificación se puede presentar en grados iguales o variables en diferentes sitios en un polipéptido dado. Los polipéptidos del invento pueden ser ramificados, por ejemplo, como resultado de ubiquitinación, y pueden ser cíclicos, con o sin ramificación. Los polipéptidos cíclicos, ramificados, y ramificados cíclicos pueden resultar a partir de procesos naturales de posttraslación o se pueden preparar mediante métodos sintéticos. Las modificaciones incluyen acetilación, acilación, ADP-ribosilación, amidación, unión covalente de flavina, unión covalente de una fracción heme, unión covalente de un nucleotido o derivado de nucleotido, unión covalente de un lípido o derivado lípido, unión covalente de fosfotidilinositol, entrecruzamiento, ciclización, formación de enlaces de disulfuro, desmetilación, formación de entrecruzamaientos covalentes, formación de cisteína, formación de piroglutamato, formilación, gama-carboxilación, glicosilación, formación de anclaje GPI (*glucosulfosfatidilinositol*), hidroxilación, yodinación, mutilación, miristilación, oxidación, pegilación, procesamiento proteolítico, fosforilación, prenilación, racemización, selenoilación, sulfatación, adición de aminoácidos mediada por ARN de transferencia a proteínas tal como arginilación, y ubiquitinación. (Ver, por ejemplo, PROTEINS –STRUCTURE AND MOLECULAR

PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New Cork (1993); POST-TRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press. New York, pgs 1-12 (1983); Seifer Meth. Enzymol. 182:626-646 (1990); Rattan, Ann. N.Y. Acad. Sci. 663:48-62 (1992)).

Las proteínas de fusión de albúmina del invento y anticuerpos que fijan una proteína terapéutica o fragmentos o variantes de la misma se pueden fusionar a secuencias marcadoras, tales como un péptido para facilitar purificación. En realizaciones preferidas, una secuencia de aminoácidos marcadores es un péptido hexa-histidina, tal como la etiqueta proporcionada en un vector pQE (QIAGEN, Inc., Eton Avenue, Chatsworth, CA, 91311), entre otros, muchos de los cuales son comercialmente disponibles. Como se describe en Gentz, Proc. Natl. Acad. Sci. USA 86:821-824 (1989), por ejemplo, hexa-histidina proporciona purificación conveniente de la proteina de fusión. Otras etiquetas peptídicas útiles para purificación incluyen, pero no están limitadas a la etiqueta "HA", la cual corresponde a un epítope derivado de la proteína hemaglutinina de influenza (Wilson, Cell 37:767 (1984) y la etiqueta "bandera".

Además, una proteína de fusión de albúmina del invento se puede conjugar a una fracción terapéutica tal como una citotoxina, ej., un agente citostático o citocidal, un agente terapéutico o un ion metálico radiactivo, ej., emisores alfa tales como, por ejemplo, 213Bi. Una citotoxina o agente citotóxico incluye cualquier agente que sea perjudicial para las células. Ejemplos incluyen: paclitaxol, citochalasina B, gramicidina D, bromuro de etidio, emetina, mitomicina, etoposido, tenoposido, vincristina, colchicina, doxorubicina, diona de dihidroxi-antracina, mitoxantrona, mitramicina, actinomicina D, 1-dehidrotestosterona, glococorticoides, procaina, tetracaina, lidocaina, propranolol, y puromicina y análogos u homólogos de las mismas. Los agentes terapéuticos incluyen pero no están limitados a antimetabolitos (ej., metotrexato, 6-mercaptopurina, 6-tioguanina, citarabina, decarbacina de 5-fluorouracilo), agentes alquilantes (ej., mecloretamina, tioepa clorambucil, melfalan, carmustina (BSNU) y lomustina (CCNU), ciclotosfamida, busulfan, dibromomanitol, estreptozotocina, mitomicina C, y cis-diclorodiamina platino (II) (DDP) cisplatina), antraciclinas (ej., daunorubicina (anteriormente daunomicina), anticuerpos (ej., dactinomicina (anteriormente actinomicina), bleomicina, mitramicina, y antramicina (AMC), y agentes anti-mitóticos (ej., vincristina y vinblastina).

Los conjugados del invento se pueden utilizar para modificar una respuesta biológica dada, el agente terapéutico o fracción farmacéutica no se considerará limitado a agentes terapéuticos químicos clásicos. Por ejemplo, la fracción farmacéutica puede ser una proteína o polipéptido que posee una actividad biológica deseada. Tales proteínas pueden incluir, por ejemplo, una toxina tal como abrina, ricino A, exotoxina pseudomonas, o tóxina de difteria; una proteina tal como factor de necrosis tumoral, alfa-interferón, beta-interferón, factor de crecimiento nervioso, factor de crecimiento derivado de plaquetas, activador de plaminogeno de tejidos, un agente apoptótico, ej., TNF-alfa, TNF-beta, AIM I (Ver, Publicación Internacional No. WO 97/33899), AIM II (Ver, Publicación Internacional No. WO 87/34911), Fas Ligand (Takahashi *Int. Immunol.* 6:1567-1574 (1994)), VEGI (Ver, Publicación Internacional No. WO 99/23105), un agente trombótico o un agente anti-angiogénico, ej., angiostatina o endostatina; o, modificadores de respuesta biológica tales como, por ejemplo, linfoquinas, interleucina-1 ("IL-1"), interleucina-2 ("IL-2"), interleucina-6 ("IL-6"), factor estimulante de colonias de granulocitos macrofogos ("GM-CSF"), factor estimulante de colonias de granulocitos ("G-CSF"), u otros factores de crecimiento. Técnicas para conjugar tal fracción terapéutica a proteínas (ej., proteínas de fusión de albúmina) son bien conocidas en el estado de la técnica.

Las proteínas de fusión de albúmina también se pueden fijar a soportes sólidos, los cuales son particularmente útiles para inmunoensayos o purificación de polipeptídicas que son unidos

por, que se unen a, o asocian con proteínas de fusión de albúmina del invento. Tales soportes sólidos incluyen pero no están limitados a vidrio, celulosa, poliacrilamida, nailon, poliestireno, cloruro de polivinilo, polipropileno.

Las proteínas de fusión de albúmina, con o sin fracción terapéutica conjugada a estas, administradas solas o en combinación con un factor citotóxicos y/o citoquina(s) se pueden utilizar como un terapéutico.

En realizaciones donde la proteína de fusión de albúmina del invento comprende solamente el dominio VH de un anticuerpo que fija una proteína terapéutica, puede ser necesario y/o deseable coexpresar la proteína de fusión con el dominio VL del mismo anticuerpo que fija una proteína Terapéutica, tal que la proteína de fusión de albúmina VH y la proteína VL se asociará (ya sea de manera covalente o no covalente) post-traslacionalmente.

En realizaciones donde la proteína de fusión de albúmina del invento comprende solamente el dominio VL de un anticuerpo que fija una proteína terapéutica, puede ser necesario y/o deseable coexpresar la proteína de fusión con el dominio VH del mismo anticuerpo que fija una proteína terapéutica, tal que la proteína de fusión de albúmina VL y la proteína VH se asociará (ya se de manera covalente o no covalente) post-traslacionalmente.

Algunos anticuerpos terapéuticos son anticuerpos biespecíficos, lo cual significa que el anticuerpo que fija una proteína terapéutica es un anticuerpo híbrido artificial que tiene dos pares de cadenas diferentes pesada/liviana y dos sitios de unión diferentes. Con el objeto de crear una proteína de fusión de albúmina correspondiente a esa proteína terapéutica, es posible crear una proteína de fusión de albúmina la cual tiene un fragmento scFv fusionado a ambos términos N y C de la fracción de proteína de albúmina. Más anti nuevamente, el scFv fusionado al extremo N correspondería a uno de los pares pesado/liviano (VH/VL) del anticuerpo original que fija una proteína terapéutica y el scFv fusionado al extremo C de albúmina correspondería al otro par liviano/pesado (VH/VL) del anticuerpo original que fija una proteína terapéutica.

El invento también proporciona derivados químicamente modificados de las proteínas de fusión de albúmina del invento los cuales pueden proporcionar ventajas adicionales tales como solubilidad, estabilidad y tiempo de circulación aumentados del polipéptido, o inmunogenicidad disminuida (ver Patente Americana No. 4.179.337). Las fracciones químicas para derivatización se pueden seleccionar a partir de polímeros solubles de agua tales como glicol de polietileno, copolímeros de glicol de etileno y glicol de propileno, carboximetilcelulosa, dextrana, alcohol polivinílico y similares. Las proteínas de fusión de albúmina se pueden modificar en posiciones aleatorias dentro de la molécula, o en posiciones predeterminadas dentro de la molécula y pueden incluir una, dos, tres, o más fracciones químicas fijadas.

El polímero puede tener cualquier peso molecular y puede ser ramificado o no ramificado. Para glicol de polietileno, el peso molecular preferido está entre 1kDa y 100 kDa aproximadamente (el término "aproximadamente" indica que en preparaciones de glicol de polietileno, algunas moléculas pesarán más, algunas menos, que el peso molecular declarado) para facilidad de manejo y preparación. Se pueden utilizar otros tamaños, dependiendo del perfil terapéutico deseado (ej., la duración de liberación sostenida deseada, los efectos, si existen, sobre actividad biológica, la facilidad de manejo, el grado o falta de antigenicidad y otros efectos conocidos del glicol de polietileno a la proteína terapéutica o análogo). Por ejemplo, el glicol de polietileno puede tener un peso molecular promedio de aproximadamente 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10.00010.500, 11.000, 11.500, 12.000, 12.500, 13.000, 13.500, 14.000, 14.500, 15.000, 15.500, 16.000, 16.500, 17, 000, 17.500, 18.000, 18.500, 19.000, 19.500, 20.000 25.000, 30.000,

35.000, 40.000, 45.000, 50.000, 55.000, 60.000, 65.000, 70.000, 75.000, 80.000, 85.000, 90.000, 95.000, o 100, 000 kDa.

Como se anotó anteriormente, el glicol de polietileno puede tener una estructura ramificada. Los glicoles de polietileno ramificados son descritos, por ejemplo, en la Patente Americana No. 5.643.575; Morpurgo *App. Biotecnol. 56:*59-72 (1996); Vorobjev, *Nucleosides Nucleotides* 18:2745-2750 (1999); y Caliceti *Bioconjug. Chem.* 10:638-646 (1999), las divulgaciones de estas son incorporadas en la presente como referencia.

Las moléculas de glicol de polietileno (u otras fracciones químicas) se deben fijar a la proteína con consideración de efectos sobre dominios funcionales o antigénicos de la proteína. Existe un número de métodos de fijación disponibles para aquellos con experiencia en el estado de la técnica, tal como por ejemplo, el método divulgado en EP 0 401 384 (acoplamiento PEG a G-CSF), incorporado en la presente como referencia; ver también Malik, Exp. Hematol. 20:1028-1035 (1992), reportando pegilación de GM-CSF utilizando cloruro de tresilo. Por ejemplo, el glicol de polietileno puede estar unido de manera covalente a través de residuos aminoácidos por medio del grupo reactivo, tal como un grupo amino o carboxilo libre. Los grupos reactivos son aquellos a los cuales una molécula de glicol de polietileno activada puede estar unida. Los residuos aminoácidos que tienen un grupo amino libre pueden incluir residuos de lisina y los residuos aminoácidos N-terminales; aquellos con un grupo carboxilo libre pueden incluir residuos de ácido aspártico, residuos de ácido glutámico y el residuo aminoácido C-terminal. Los grupos de sulfhidrilo también se pueden utilizar como un grupo reactivo para fijar las moleculas de glicol de polietileno. Para propósitos terapéuticos se prefiere la fijación en un grupo amino, tal como fijación en el término N o grupo de lisina.

Como se indicó anteriormente, el glicol de polietileno se puede fijar a proteínas a través de unión con cualquier número de residuos aminoácidos. Por ejemplo, el glicol de polietileno puede estar ligado a proteínas a través de enlaces covalentes a lisina, histidina, ácido aspártico, ácido glutámico, o residuos de cisteina. Se puede emplear una o más químicas de reacción para fijar glicol de polietileno a residuos aminoácidos específicos (ej., lisina, histidina, ácido aspártico, ácido glutámico, o cisteina) de la proteína o a más de un tipo de residuo aminoácido (ej., lisina, histidina, ácido aspártico, ácido glutámico, cisteina y combinaciones de los mismos) de la proteína.

Se puede desear específicamente proteínas químicamente modificadas en el término N. Utilizando glicol de polietileno como una ilustración de la presente composición, se puede seleccionar a partir una variedad de moléculas de glicol de polietileno (mediante peso molecular, ramificación, etc.), la proporción de moléculas de glicol de polietileno a moléculas de proteína (polipéptido) en la mezcla de reacción, el tipo de reacción de pegilación a ser realizada, y el método para obtener la proteína N-terminalmente pegilada seleccionada. El método para obtener la preparación N-terminalmente pegilada (es decir, separando esta fracción de otras fracciones monopegiladas si es necesario) puede ser mediante purificación del material N-terminalmente pegilado desde una población de moléculas de proteina pegiladas. Las proteínas selectivas qumicamente modificadas en la modificación del N-término se puede lograr mediante alquilación reductiva la cual explota la reactividad diferencial de diferentes tipos de grupos amino primarios (lisina versus el N-término) disponible para derivatización en una proteína particular. Bajo las condiciones de reacción apropiadas, se logra derivatización sustancialmente selectiva de la proteína en el N-término con un grupo carbonilo que contiene el polímero.

Como se indicó anteriormente, la pegilación de las proteínas de fusión de albúmina del invento se puede realizar mediante cualquier número de medios. Por ejemplo, el glicol de polietileno se puede fijar a la proteína de fusión de albúmina de manera directa o mediante un

enlazador interventor. Sistemas libres enlazadores para fijar glicol de polietileno a proteínas son descritos en Delgado, Crit. Rev. Thera. Drug Carrier Sys. 9:249-304 (1992); Francis, Intern. J. of Hematol 68:1-18 (1998); Patente Americana No. 4.002.531; Patente Americana No. 5.349.052; WO 95/06058; y WO 98/32466, las divulgaciones de cada una de estas es incorporada en la presente como referencia.

Un sistema para fijar glicol de polietileno directamente a residuos aminoácidos de proteínas sin enlazador de intervención emplea MPEG tresilado, el cual es producido mediante la modificación de monometoxi polietilenglicol (MPEG) utilizando tresilcloruro (CISO2CH2CF3). Ante reacción de proteína con MPEG tresilado, el glicol de polietileno es fijado directamente a grupos amina de la proteína. Por lo tanto, el invento incluye conjugados de proteina-glicol de polietileno producidos al reaccionar proteínas del invento con una molécula de glicol de polietileno que tiene un grupo 2,2,2-trifluoroetano sulfonilo.

El glicol de polietileno también se puede fijar a proteínas utilizando un número de diferentes enlazadores interventores. Por ejemplo, la Patente Americana No. 5.612.460, la divulgación de ésta es incorporada en la presente como referencia, divulga enlazadores de uretano para conectar glicol de polietileno a proteínas. Los conjugados de proteína-glicol de polietileno en donde el glicol de polietileno está fijado a la proteína mediante un enlazador, también se pueden producir mediante reacción de proteínas con compuestos tales como MPEG-succinimidilsuccinato, MPEG activado con 1,1'-carbonildiimidazol, MPEG-2,4,5-tricloropenilcarbonato, MPEG-p-nitrofenocarbonato, y varios derivados MPEG-succinato. Un número de derivados de glicol de polietileno adicionales y químicas de reacción para fijar glicol de polietileno a proteínas son descritos en la Publicación Internacional No. WO 98/32455, la divulgación de esta es incorporada en la presente como referencia. Los productos de proteínas de pegilación producidos utilizando las químicas de reacción divulgadas en la presente son incluidos dentro del alcance del invento.

El número de fracciones de glicol de polietileno unidas a cada proteína de fusión del invento (es decir, el grado de sustitución) también puede variar. Por ejemplo, las proteínas pegiladas del invento pueden estar ligadas, en promedio a 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 17, 20 o más moleculas de glicol de polietileno. De manera similar, el grado promedio de sustitución dentro de rangos tales como 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, o 18-20 fracciones de glicol de polietileno por molécula de proteína. Los métodos para determinar el grado de sustitución son discutidos por ejemplo, en Delgado, Crit, Rev. Thera, Drug Carrier Sys. 9:249-304 (1992).

Los polipéptidos del invento se pueden recuperar y purificar a partir de síntesis química y cultivos de células recombinantes mediante métodos estándaresn los cuales incluyen, pero no están limitados a, sulfato de amonio o precipitación de etanol, extracción de ácidos, cromatografía de intercambio aniónico o catiónico, cromatografía de fosfocelulosa, cromatografía de interacción hidrofóbica, cromatografía de afinidad, cromatografía de hidroxilapatita y cromatografía de lectina. Más preferiblemente, cromatografía líquida de alto desempeño ("HPLC") es empleada para purificación. Técnicas bien conocidas para replegamiento de proteínas pueden ser empleadas para regenerar conformación activa cuando el polipéptido es desnaturalizado durante aislamiento y/o purificación.

La presencia y cantidad de proteínas de fusión de albúmina del invento se puede determinar utilizando ELISA, un inmunoensayo bien conocido de el estado de la técnica. En un protocolo ELISA que sería útil para detectar cuantificar proteínas de fusión de albúmina del invento, comprende los pasos de recubrir una placa ELISA con un anticuerpo de albúmina sérica anti-humano, bloqueo de la placa para prevenir unión no específica, lavado de la placa ELISA,

agregando una solución que contiene la proteína de fusión de albúmina del invento (en una o más concentraciones diferentes), agregando un anticuerpo específico de proteína anti-terapéutica acoplado a un rotulo detectable (tal como se describe en la presente o de otra manera se conoce en el estado de la técnica), y detectando la presencia del anticuerpo secundario. En una versión alternativa de este protocolo, la placa ELISA se puede recubrir con el anticuerpo específico de proteína anti-Terapéutica y el reactivo secundario rotulado puede ser el anticuerpo específico anti-humano.

Usos de los Polinucleotidos

Cada uno de los polinucleotidos identificados en la presente se pueden utilizar en numerosas formas como reactivos. La siguiente descripción se debe considerar de ejemplo y utiliza técnicas conocidas.

Los polinucleotidos del presente invento son útiles para producir las proteínas de fusión de albúmina de invento. Como se describe en detalle a continuación, los polinucleotidos del invento (que codifican proteínas de fusión de albúmina) se pueden utilizar en métodos de ADN recombinante útiles en ingeniería genética para fabricar células, líneas celulares, o tejidos que expresen la proteína de fusión de albúmina codificada por los polinucleotidos que codifican proteínas de fusión de albúmina del invento.

Los polinucleotidos del presente invento también son útiles en terapia genética. Una meta de la terapia genética es insertar un gen normal en un organismo que tiene un gen defectuoso, en un esfuerzo por corregir el defecto genético. Los polinucleotidos divulgados en el presente invento ofrecen un medio de enfocarse en tales defectos genéticos en una manera altamente exacta. Otra meta es insertar un nuevo gen que no estaba presente en el genoma huesped, produciendo así un nuevo rasgo en la célula huesped. Ejemplos no limitantes adicionales de métodos de terapia genética contemplados por el presente invento son descritos de manera más completa en otra parte en la presente (ver, ej., las secciones rotuladas "Terapia Genética", y los Ejemplos 61 y 62).

Usos de los polipéptidos

Cada uno de los polipéptidos identificados en la presente se puede utilizar en numerosas formas. La siguiente descripción se debe considerar de ejemplo y utiliza técnicas conocidas.

Las proteínas de fusión de albúmina del invento son útiles para proporcionar sondas inmunológicas para identificación diferencial de los tejidos(s) (ej., ensayos de inmunohistoquímica tales como, por ejemplo, inmunoperoxidasa ABC (Hsu, J. Histochem. Cytochem. 29:577-580 (1981) o tipos celulares (ej., ensayos de inmunohistoquímica).

Las proteínas de fusión de albúmina se pueden utilizar para probar niveles de polipéptidos en una muestra biológica utilizando métodos inmunohistológicos clásicos conocidos por aquellos con experiencia del estado de la técnica (ej., ver Jalkanen, J. Cell. Biol. 101:976-985); Jalkanen, J. Cell. Biol. 105:3087-3096 (1987)). Otros métodos útiles para detectar expresión genética de proteínas incluyen inmunoensayos, tales como el ensayo con inmunosorbente ligado a enzima (ELISA) y el radioinmunoensayo (RIA). Rotulos de ensayos apropiados son conocidos en el estado de la técnica e incluyen rotulos de enzimas, tales como yodo (^{121}I, ^{123}I, ^{125}I, ^{131}I), carbono (^{14}C), azufre (^{35}S), tritio (^{3}H), indio (^{111}In, ^{112}In, ^{113m}In, ^{115m}In), tecnetio (^{99}Tc, ^{99m}Tc), talio (^{201}Ti), galio (^{68}Ga, ^{67}Ga), paladio(^{103}Pd), molibdeno (^{99}Mo), xenon (^{133}Xe), fluor(^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, y ^{97}Ru; rótulos luminiscentes, tales como luminol; y rótulos fluorescentes, tales como fluoresceína y rodamina, y biotina.

Las proteínas de fusión de albúmina del invento también se pueden detectar in vivo mediante creación de imágenes. Los rotulos o marcadores para creación de imágenes *in vivo* de proteínas incluyen aquellos detectables mediante radiografia X, resonancia magnética nuclear (NMR) o relajación de espín electrónico (ESO). Para radiografia-X, los rotulos apropiados incluyen radioisotopos tales como bario o cesio, los cuales emiten radiación detectable pero no manifiestamente perjudicial para el sujeto. Los marcadores adecuados para NMR y ESR incluyen aquellos con un espín característico detectable, tal como deuterio, los cuales se pueden incorporar en la proteína de fusión de albúmina mediante rotulación de nutrientes dados en una línea celular que expresa la proteína de fusión de albúmina del invento.

Una proteína de fusión de albúmina la cual se ha rotulado con una fracción de imágenes detectable apropiada, tal como un radioisotopo (por ejemplo, ^{123}I, ^{112}In, ^{99m}Tc, (^{125}I, ^{131}I, ^{123}I, ^{121}I), carbono (^{14}C), azufre (^{35}S), tritio (^{3}H), indio (^{111}In, ^{112}In, ^{113m}In, ^{115m}In), y tecnetio (^{99}Tc, ^{99m}Tc), talio (^{201}Ti), galio (^{68}Ga, ^{67}Ga), paladio(^{103}Pd), molibdeno (^{99}Mo), xenon (^{133}Xe), fluor(^{18}F, ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, y ^{97}Ru), una sustancia radio-opaca, o un material detectable mediante resonancia magnética nuclear, es introducido (por ejemplo, de manera parenteral, subcutánea o intraperitoneal) en el mamífero a ser examinado para trastorno del sistema inmune. Se entenderá en el estado de la técnica que el tamaño del sujeto y el sistema de imágenes utilizado determinará la cantidad de fracción de imágenes necesaria para producir imágenes de diagnóstico. En el caso de una fracción de radioisotopo, para un sujeto humano, la cantidad de radioactividad inyectada normalmente oscilará entre 5 y 20 milicuries de 99mTc. La proteína de fusión de albúmina rotulada luego preferencialmente se acumulará en ubicaciones en el cuerpo (ej., órganos, células, especios extracelulares o matrices) donde uno o más receptores, ligandos o sustratos (correspondientes a aquel de la proteína terapéutica utilizada para preparar la proteína de fusión de albúmina del invento) son localizados. Alternativamente, en el caso donde la proteína de fusión de albúmina comprende al menos un fragmento o variante de un anticuerpo Terapéutico, la proteína de fusión de albúmina preferencialmente luego se acumulará en las ubicaciones en el cuerpo (ej., órganos, células, espacios extracelulares o matrices) donde los polipéptidos/epítopes correspondientes a aquellos ligados por el anticuerpo Terapéutico (utilizados para preparar la proteína de fusión de albúmina del invento) son localizados. La creación de imágenes tumorales *in vivo* se describen en S.W. Burchiel, "Inmunofarmacocínetica de Anticuerpos Radiorotulados y Sus Fragmentos" (Capítulo 13 *in Tumor Imaging: The Radiochemical Detection of Cáncer,* S.W. Burchiel y B.A. Rhodes, eds., Masson Publishing Inc. (1982)). Los protocolos descritos en la presente se podrían modificar de manera fácil por alguien con experiencia en el estado de la técnica para utilizar con las proteínas de fusión de albúmina del invento.

En una realización, el invento proporciona un método para la liberación específica de proteínas de fusión de albúmina del invento a células mediante administración de proteínas de fusión de albúmina del invento (ej., polipéptidos codificados mediante polinucleotidos que codifican proteínas de fusión de albúmina del invento y/o anticuerpos) que están asociadas con polipéptidos heterólogos o ácidos nucleicos. En un ejemplo, el invento proporciona un método para liberar una proteína terapéutica en la célula objetivo. En otro ejemplo, el invento proporciona un método para liberar un ácido nucleico de hebra simple (ej., antisentido o ribosomas) o ácido nucleico de doble hebra (ej., ADN que se puede integrar en el genóma de la célula o replicar episomalmente y que se puede transcribir) en la célula objetivo.

En otra realización, el invento proporciona un método para destrucción específica de células (ej., destrucción de células tumorales) mediante administración de proteínas de fusión de albúmina del invento en asociación con toxinas o profármacos citotóxicos.

Por "toxina" se entiende uno o más compuestos que fijan y activan sistemas receptores citotóxicos endógenos, radioisótopos, holotoxinas, toxinas modificadas, subunidades catalíticas de toxinas, o cualquier molécula o enzima normalmente no presente en o sobre la superficie de una célula que bajo las condiciones definidas produce la muerte de la célula. Las toxinas que se pueden utilizar según los métodos del invento incluyen, pero no están limitadas a radioisótopos conocidos en el estado técnica, compuestos tales como, por ejemplo, anticuerpos (o fijación de complemento que contienen porciones de los mismos) que se unen a un sistema efector citotóxico endógenos inherente o inducido, timidina quinasa, endonucleasa, ARNasa, toxina alfa, ricina, abrina, exotoxina A de *Pseudomonas*, toxina de difteria, saporina, momordina, gelonina, proteína antiviral *pokeweed*, toxina del cólera y alpha-sarcina. "toxina" también incluye un agente citostático o citocidal, un agente terapéutico o un ión metálico radioactivo, ej., emisores alfa tal como, por ejemplo, 213Bi, u otros radioisotopos tales como, por ejemplo, ^{103}Pd, ^{133}Xe, ^{131}I, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{35}S, ^{90}Y, ^{153}Sm, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{113}Sn, ^{90}Ytrio, ^{117}Estaño, ^{186}Renio, ^{166}Holmio, y ^{188}Renio; rotulos luminiscentes tales como luminol; y rotulos fluorescentes, tales como fluoresceína y rodamina, y biotina. En una realización específica, el invento proporciona un método para destrucción específica de células (ej., la destrucción de células tumorales) mediante administración de polipéptidos del invento o anticuerpos del invento en asociación con el radioisotopo ^{90}Y. En otra realización específica, el invento proporciona un método para la destrucción específica de células (ej., la destrucción de células tumorales) mediante la administración de polipéptidos del invento o anticuerpos del invento en asociación con el radioisotopo ^{111}In. En una realización específica adicional, el invento proporciona un método para la destrucción específica de células (ej., la destrucción de células tumorales) mediante la administración de polipéptidos del invento o anticuerpos del invento en asociación con el radioisótopo ^{131}I.

Técnicas conocidas en el estado de la técnica se pueden aplicar para rotular polipéptidos del invento. Tales técnicas incluyen, pero no están limitadas al uso de agentes de conjugación bifuncionales (ver ej., Patentes Americanas No. 5.756.065; 5.714.631; 5.696.239; 5.696.239; 5.652.361; 5.505.931; 5.489.425; 5.435.990; 5.428.139, 5.342.604; 5.274.119; 4.994.560; y 5.808.003; las cuales son incorporadas en la presente como referencia en su totalidad).

Las proteínas de fusión de albúmina del presente invento son útiles para diagnosis, tratamiento, prevención y/o prognosis de varios trastornos en mamíferos, preferiblemente humanos. Tales trastornos incluyen, pero no están limitados a aquellos descritos bajo el titulo de sección "Actividades Biológicas", a continuación.

Por lo tanto, el invento proporciona un método de diagnóstico de un trastorno, el cual involucra (a) prueba del nivel de expresión de un cierto polipéptido en células o fluido corporal de un individuo utilizando una proteína de fusión de albumina del invento; y (b) comparación del nivel de expresión polipeptido probado con un nivel de expresión polipéptido estándar, mediante la cual un aumento o disminución en el nivel de expresión polipeptido probado en comparación con el nivel de expresión estándar es indicativo de un trastorno. Con respecto a cáncer, la presencia de una cantidad relativamente alta de transcripto en tejido biopsiado de un individuo puede indicar una predisposición al desarrollo de la enfermedad, o puede proporcionar un significado para detectar la enfermedad antes de la aparición de síntomas clínicos actuales. Una diagnosis más definitiva de este tipo puede permitir a profesionales de la salud emplear medidas preventivas o tratamiento agresivo temprano evitando así el desarrollo o avance adicional del cáncer.

Además, las proteínas de fusión de albúmina del presente invento se pueden utilizar para tratar a prevenir enfermedades o condiciones tales como, por ejemplo, trastornos neurales,

trastornos del sistema inmune, trastornos musculares, trastornos reproductivos, trastornos gastrointestinales, trastornos pulmonares, trastornos cardiovasculares, trastornos renales, trastornos proliferativos, y/o enfermedades y condiciones cancerosas. Por ejemplo, a los pacientes se puede administrar un polipéptido del presente invento en un esfuerzo por reemplazar niveles ausentes o disminuidos del polipéptido (ej., insulina), para suplementar niveles ausentes o disminuidos de un polipéptido diferente (ej., hemoglobina S por hemoglobina B, SOD, catalasa, proteínas de reparación de ADN), para inhibir la actividad de un polipéptido (ej., un oncogeno o supresor tumoral), para activar la actividad de un polipéptido (ej., mediante unión a un receptor), para reducir la actividad de un receptor unido a membrana al competir con éste por ligando libre (ej., receptores TNF solubles utilizados para reducir inflamación), para averigurar una respuesta deseada (ej., inhibición de crecimiento de vasos sanguineos, aumento de la respuesta inmune a tejidos o células proliferativos).

En particular, las proteínas de fusión de albúmina que comprenden al menos un fragmento o variante de un anticuerpo terapéutico también se pueden utilizar para tratar una enfermedad (tal como se describió anteriormente, y en otra parte en la presente). Por ejemplo, la administración de una proteína de fusión de albumina que comprende al menos un fragmento o variante de un anticuerpo Terapéutico puede unir, y/o neutralizar el polipéptido al cual el anticuerpo Terapéutico utilizado para preparar la proteina de fusión de albúmina, especificamente se une, y/o reducir sobreproducción del polipéptido al cual el anticuerpo terapéutico utilizado para preparar la proteína de fusión de albúmina específicamente se une. De manera similar, la administración de una proteína de fusión de albúmina comprendiendo al menos un fragmento o variante de un anticuerpo terapéutico puede activar el polipéptido al cual el anticuerpo terapéutico utilizado para preparar la proteína de fusión de albúmina específicamente se une, mediante unión a polipéptido unido a una membrana (receptor).

Como mínimo, las proteínas de fusión de albúmina del invento del presente invento se pueden utilizar como marcadores de peso molecular sobre geles SDS-PAGE o sobre columnas de filtración de geles de tamices moleculares utilizando métodos bien conocidos por aquellos con experiencia en el estado de la técnica. Las proteínas de fusión de albúmina del invento también se pueden utilizar para elevar anticuerpos, los cuales a su vez se pueden utilizar para medir expresión de proteínas de la proteína Terapéutica, proteína de albumina, y/o la proteína de fusión de albumina del invento a partir de una célula recombinante, como una forma de evaluar la transformación de la célula huesped, o en una muestra biológica. Además, las proteínas de fusión de albúmina del presente invento se pueden utilizar para probar las actividades biológicas descritas en la presente.

Ensayos Diagnósticos

Los compuestos del presente invento son útiles para diagnosis, tratamiento, prevención y/o prognosis de varios trastornos en mamíferos, preferiblemente humanos. Tales trastornos incluyen, pero no están limitadas a aquellos descritos para cada proteína terapéutica en la correspondiente fila de la tabla 1 y en la presente bajo los títulos de sección "actividad inmune", "trastornos relacionados con sangre," "trastornos hiperproliferativos", "desórdenes renales", "desórdenes cardiovasculares," "desórdenes respiratorios," "actividad anti-antiogénesis," "enfermedades a nivel celular", "Curación de heridas y proliferación celular epitelial", "actividad neuronal y enfermedades neurológicas", "trastornos endocrinos," "trastornos del sistema reproductivo", "enfermedad infecciosa", "regeneración", y/o "trastornos gastrointestinales," *infra.*

Para un número trastornos, los niveles sustancialmente alterados (aumentados o disminuidos) de expresión genética se pueden detectar en tejidos, células o fluidos corporales (ej., suero, plasma, orina, semen, fluido sinovial o fluido espinal) tomados de un individuo que tiene

tal trastorno, con relación a un nivel de expresión genética "estándar", es decir, el nivel de expresión en tejidos o fluidos corporales de un individuo que no tiene tal trastorno. Por lo tanto, el invento proporciona un método de diagnóstico útil durante diagnosis de un trastorno, el cual involucra la medición del nivel de expresión del gen que codifica un polipéptido en tejidos, células o fluido corporal de un individuo y compara el nivel de expresión genética medida con un nivel de expresión genética estándar, mediante lo cual un aumento o disminución en el nivel de expresión genética en comparación con el estándar es indicativo de un trastorno. Estos ensayos diagnósticos se pueden realizar in vivo o in vitro, tal como, por ejemplo, sobre muestras de sangre, tejidos de biopsia o tejidos de autopsia.

El presente invento también es útil como indicador prognóstico, mediante el cual los pacientes que exhiben expresión genética aumentada o deprimida experimentarán un resultado clínico peor.

Por "probar el nivel de expresión del gen que codifica un polipéptido" se entiende la medición cualitativa o cuantitativa o estimación del nivel de un polipéptido particular (ej., un polipéptido que corresponde una proteína terapéutica divulgada en la tabla 1) o el nivel de mARN que codifica el polipéptido del invento en la primera muestra biológica ya sea directamente (ej., al determinar o estimar el nivel de proteínas absoluto o nivel mARN) o de manera relativa (ej., al comparar con el nivel polipéptido o nivel mARN en una segunda muestra biológica). Preferiblemente, el nivel de expresión polipéptida o nivel mARN en la primera muestra biológica es medido o estimado y comparado con un nivel polipéptido estándar o nivel mARN, el estándar es tomado a partir de una segunda muestra biológica obtenida de un individuo que no tiene el trastorno o es determinado al promediar niveles de una población de individuos que no tienen el trastorno. Como se apreciará en el estado de la técnica, una vez el nivel polipéptido estándar o nivel mARN es conocido, se puede utilizar repetidamente como un estándar para comparación.

Por "muestra biológica" se entiende cualquier muestra biológica obtenida de un individuo, línea celular, cultivo de tejidos, u otra fuente que contiene polipéptidos del invento (incluyendo porciones de los mismos) o mARN. Como se indicó, las muestras biológicas incluyen fluidos corporales (tales como sueros, plasma, orina, fluido sinovial y fluido espinal) y fuentes de tejidos encontrados para expresar la longitud total o fragmentos de los mismos de un polipéptido o mARN. Los métodos para obtener biopsias de tejidos y fluidos corporales de mamíferos son bien conocidos en el estado de la técnica. Donde la muestra biológica es para incluir mARN, una biopsia de tejidos en la fuente preferida.

El ARN celular total se puede aislar a partir de una muestra biológica utilizando cualquier técnica apropiada tal como el método de guanidinio-tiocianato-fenol-cloroformo descrito en Chomczybski y Sacchi, Anal.Biochem. 162:156-159 (1987). Los niveles de mARN que codifica los polipéptidos del invento luego son probados utilizando cualquier método apropiado. Estos incluyen análisis Northern blot, mapeo de nucleasa S1, la reacción de cadena de polimerasa (PCR), transcripción inversa en combinación con la reacción de cadena de polimerasa (RT-PCR), y transcripción inversa en combinación con la reacción de cadena de ligasa (RT-LCR).

El presente invento también se relaciona con ensayos diagnósticos tales como ensayos cuantitativos y diagnósticos para detectar niveles de polipéptidos que se unen a, están unidos por, o se asocian con proteínas de fusión de albúmina del invento, en una muestra biológica (ej., células y tejidos), incluyendo determinación de niveles normales y anormales de polipéptidos. Así, por ejemplo, un ensayo diagnóstico según el invento para detectar expresión anormal de polipéptidos que se unen a, son unidos por, o se asocian con proteínas de fusión de albúmina en comparación con muestras de tejidos de control normales, se puede utilizar para detectar la presencia de tumores. Técnicas de ensayos que se pueden utilizar para determinar niveles de

polipéptidos que se unen a, están unidos por, o se asocian con proteínas de fusión de albúmina del presente invento en una muestra derivada a partir de un huesped, son bien conocidas por aquellos con experiencia en estado técnica. Tales métodos de ensayo incluyen radioinmunoensayos, ensayos de unión competitiva, análisis Western Blot y ensayos ELISA. Probar niveles polipéptidos en una muestra biológica puede ocurrir utilizando cualquier método conocido.

La prueba de niveles polipeptidos en una muestra biológica puede ocurrir utilizando una variedad de técnicas. Por ejemplo, la expresión polipéptida en tejidos se puede estudiar con métodos inmunohistoquímicos clásicos (Jalkanen, J. Cell. Biol. 101:976-985; Jalkanen, J. Cell. Biol. 105:3087-3096 (1987)). Otros métodos útiles para detectar expresión genética polipeptida incluyen inmunoensayos, tales como ensayo con inmunosorbente ligado a enzima (ELISA) y el radioinmunoensayo (RIA). Rotulos de ensayos de anticuerpos adecuados son conocidos en el estado de la técnica e incluyen rotulos de enzimas, tales como, oxidasa de glucosa, y radioisotopos, tales como yodo ^{125}I, ^{121}I), carbono (^{14}C) azufre (^{35}S), tritio(^{3}H), indio(^{112}In), y tecnetio (^{99m}Tc), y rotulos fluorescentes, tales como fluoresceina y rodamina, y biotina.

El tejido o tipo celular a ser analizado generalmente incluye aquellos los cuales son conocidos, o se sospecha expresan el gen de interes (tal como, por ejemplo, cáncer). Los métodos de aislamiento de proteínas empleados en la presente pueden, por ejemplo, ser tales como aquellos descritos en Harlow y Lane (Harlow, E. y Lane, D., 1988, "Anticuerpos: Un Manual de Laboratorio", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, el cual se incorpora en la presente como referencia en su totalidad. Las células aisladas se pueden derivar a partir de cultivo celular o de un paciente. El análisis de células tomadas del cultivo puede ser un paso necesario en la evaluación de las células que se pueden utilizar como parte de una técnica genética basada en células o, alternativamente, para probar el efecto de los compuestos sobre la expresión del gen.

Por ejemplo, las proteínas de fusión de albúminas se pueden utilizar para detectar cuantitativamente o cualitativamente la presencia de polipéptidos que se unen a, están unidos por, o se asocian con proteínas de fusión de albúmina del presente invento. Esto se puede realizar, por ejemplo, mediante técnicas de inmunofluorescencia empleando una proteína de fusión de albúmina rotulada de manera fluorescente acoplada con detección microscópica de luz, citométrica de flujo, o fluorométrica.

En una realización preferida, las proteínas de fusión de albúmina que comprenden al menos un fragmento variante del anticuerpo que específicamente une al menos una proteína terapéutica divulgada en la presente (ej., las proteínas terapéuticas divulgadas en la tabla 1) o de otra manera conocidas en el estado de la técnica se pueden utilizar para detectar de manera cuantitativa o cualitativa la presencia de productos genéticos o variantes conservadas o fragmentos peptídicos de los mismos. Esto se puede lograr, por ejemplo, mediante técnicas de inmunofluorescencia empleando un anticuerpo rotulado de manera fluorescente acoplado con detección microscópica de luz, citométrica de flujo o fluorométrica.

Las proteínas de fusión de albúmina del presente invento pueden, adicionalmente, ser empleadas de manera histológica, como en inmunofluorescencia, microscopia de inmunoelectrones o ensayos no inmunológicos, para detección in situ de polipéptidos que se unen a, están unidos por, o se asocian con una proteína de fusión de albúmina del presente invento. La detección in situ se puede lograr al retirar un espécimen histológico de un paciente, y aplicar a éste un anticuerpo rotulado o polipéptido del presente invento. Las proteínas de fusión de albúmina preferiblemente son aplicadas extendiendo las proteínas de fusión de albúmina rotundas sobre una muestra biológica. Mediante el uso de tal procedimiento, es posible determinar no solo la presencia de los polipéptidos que se unen a, están unidos por, o se asocian con proteínas de

fusión de albúmina, sino también su distribución en el tejido examinado. Utilizando el presente invento, aquellos con experiencia común en el estado de la técnica percibirán fácilmente que cualquiera de una prioridad de métodos histológicos (tales como procedimientos de teñido) puede ser modificado para lograr tal detección in situ.

Inmunoensayos y no inmunoensayos que detectan péptidos que se unen a, están unidos por, o se asocian con proteínas de fusión de albúmina típicamente comprenderán incubación de una muestra, tal como un fluido biológico, un extracto de tejido, células recien cosechadas, lisados de células que han sido incubados en cultivo celular, en presencia de un anticuerpo detectablemente rotulado capaz de unir productos genéticos o variantes conservadas o fragmentos peptídicos que los mismos, y detectar el anticuerpo unido mediante cualquiera de un número de técnicas bien conocidas en el estado de la técnica.

La muestra biológica puede ser llevada al contacto con e inmovilizada sobre un soporte de fase sólida o vehículo tal como nitrocelulosa, u otro soporte sólido el cual es capaz de inmovilizar células, partículas celulares o proteínas solubles. Luego el soporte se puede lavar con amortiguadores apropiados seguido por tratamiento con la proteína de fusión de albúmina detectablemente rotulada del invento. El soporte de fase sólida luego puede ser lavado con el amortiguador una vez más para retirar el anticuerpo o polipéptido sin unir. Opcionalmente el anticuerpo es subsecuentemente rotulado. La cantidad de rótulo unido sobre el soporte sólido luego se puede detectar mediante medios convencionales.

Por "vehículo o soporte de fase sólida" se entiende cualquier soporte capaz de unir un péptido (ej., una proteína de fusión de albúmina, o polipéptido que fija, está unido por, o se asocia con una proteína de fusión de albúmina del invento.) Soportes o vehículos bien conocidos incluyen vidrio, poliestireno, polipropileno, polietileno, dextrana, nailon, amilasas, celulosas naturales y modificadas, poliacrilamidas, gabros, y magnetita. La naturaleza de vehículo puede ser soluble en alguna medida o insoluble para propósitos del presente invento. El material de soporte virtualmente puede tener cualquier configuración estructural posible siempre que la molécula acoplada sea capaz de unirse a un polipéptido. Así, la configuración de soporte puede ser esférica, como una perla, o cilíndrica, como en la superficie interior de un tubo de ensayo, o la superficie exterior de un agitador. Alternativamente, la superficie puede ser plana tal como una lámina, franja de prueba, etc. Los soportes preferidos incluyen perlas de poliestireno. Aquellos con experiencia en esta técnica conocerán muchos otros vehículos apropiados para unión de anticuerpos o antígenos, o serán capaces de averiguar los mismos mediante el uso de experimentación común.

La actividad de unión de una porción dada de proteína de fusión de albúmina se puede determinar según métodos bien conocidos. Aquellos con experiencia en el estado de la técnica podrán determinar condiciones de prueba óptimas y operativas para cada determinación empleando experimentación común.

Además de evaluar niveles de polipéptidos en una muestra biológica obtenida de un individuo, el polipéptido también se puede detectar *in vivo* mediante la creación de imágenes. Por ejemplo, en una realización del invento, las proteínas de fusión de albúmina del invento se utilizan para crear imágenes de células enfermas o neoplásicas.

Los rótulos o marcadores para creación de imágenes *in vivo* de proteínas de fusión de albúmina del invento incluyen aquellos detectables mediante radiografía-X, NMR, MRI, barridos-CAT o ESR. Para radiografía-X, los rótulos adecuados incluyen radioisótopos tales como bario o cesio, los cuales emiten radiación detectable pero no son manifiestamente perjudiciales para el sujeto. Marcadores apropiados para NMR y ESR incluyen aquellos con un espín característico detectable, tal como deuterio, los cuales se pueden incorporar en la proteína

de fusión de albumina al rotular nutrientes de una línea celular (o cepa bacteriana o de levadura) diseñada.

Adicionalmente, se pueden administrar proteínas de fusión de albúmina del invento cuya presencia puede ser detectada. Por ejemplo, las proteínas de fusión de albúmina del invento rotuladas con un compuesto radioopaco u otro compuesto apropiado se pueden administrar y visualizar *in vivo*, como se discutió anteriormente para anticuerpos rotulados. Además, tales polinucleotidos se pueden utilizar para procedimientos diagnósticos in vitro.

Un anticuerpo específico a polipéptido o fragmento de anticuerpo el cuál ha sido rotulado con una fracción de imágenes detectable apropiada, tal como un radioisotopo (por ejemplo, ^{131}I, ^{112}In, ^{99m}Tc), una sustancia radioopaca, o un material detectable mediante resonancia magnética nuclear, es introducido (por ejemplo, de manera parenteral, subcutanea o intraperitoneal) en el mamífero a ser examinado para un trastorno. Se entenderá en el estado de la técnica que el tamaño del sujeto y sistema de creación de imágenes utilizadas determinará la cantidad de fracción de imágen necesaria para producir imágenes de diagnóstico. En el caso de una fracción de radioisótopo, para un sujeto humano, la cantidad de radiactividad inyectada normalmente oscilará entre 5 y 20 milicuries aproximadamente de ^{99m}Tc. La proteína de fusión de albúmina rotulada preferencialmente luego se acumulará en locaciones en el cuerpo que contengan un polipéptido u otra sustancia que se une a, está unida por o se asocia con una proteína de fusión de albúmina del presente invento. La creación de imágenes tumorales *in vivo* se describe en S.W. Burchiel, "Inmunofarmacocínetica de Anticuerpos Radiorotulados y Sus Fragmentos" (Capítulo 13 *in Tumor Imaging: The Radiochemical Detection of Cáncer,* S.W. Burchiel y B.A. Rhodes, eds., Masson Publishing Inc. (1982)).

Una de las formas en las cuales una proteína de fusión de albúmina del presente invento se puede rotular de manera detectable es mediante enlace de la misma a una enzima reportera y utilizando el producto ligado en un inmunoensayo de enzima (EIA) (Voller, A., "El Ensayo con Inmunosorbente Ligado a Enzima (ELISA)", 1978, Diagnostic Horizons 2:1-7, Microbiological Associates Quarterly Publication, Walkersville, MD); Voller, *J. Clin. Pathol.* 31:507-520 (1978); Butler, J.E., *Meth. Enzymol.* 73:482-523 (1981); Maggio, E. (ed), 1980, Enzyme Immnunoassay, CRC Press, Boca Raton, FL,; Ishikawa, E. (eds.) 1981, Enzyme Immnunoassay, Kgaku Shoin, Tokio). La enzima reportera que está unida al anticuerpo reaccionará con un sustrato apropiado, preferiblemente un sustrato cromogénico, en una forma tal para producir una fracción química la cual puede ser detectada, por ejemplo, mediante medios espectrofotométricos, fluorimétricos o visuales. Las enzimas reporteras las cuales se pueden utilizar para rotular detectablemente el anticuerpo incluyen, pero no están limitadas a malato deshidrogenasa, nucleasa estafilococal, isomerasa delta-5-esteroide, alcohol deshidrogenasa de levadura, alfa-glicerolfosfato, deshidrogenasa, triosa fosfato isomerasa, peroxidasa de rábano, fosfatasa alcalina, asparaginasa, glucosa oxidasa, beta-galactosidasa, ribonucleasa, ureasa, catalasa, deshidrogenasa de glucosa-6-fosfato, glucoamilasa y acetilcolinesterasa. Adicionalmente, la detección se puede lograr mediante métodos colorimétricos los cuales emplean un sustrato cromogénico para la enzima reportera. La detección también se puede lograr mediante comparación visual de la medida de reacción enzimática de un sustrato en comparación con estándares preparados de manera similar.

Las proteínas de fusión de albúmina también se pueden radiorotular y utilizar en cualquiera de una variedad de otros inmunoensayos. Por ejemplo, mediante rotulación radioactiva de las proteínas de fusión que albúmina, es posible utilizar las proteínas de fusión de albúmina en un radioinmunoensayo (RIA) (ver, por ejemplo, Weintraub, B. Principles of Radioimmnucoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, la cual se incorpora en la presente como referencia). El isotopo

radioactivo se puede detectar mediante medios incluyendo, pero sin limitar a, un contador gama, un contador de centelleo, o autoradiografía.

Adicionalmente, las moléculas quelantes, son conocidas en el estado de la técnica y se pueden utilizar para rotular las proteínas de fusión de albúmina. Las moléculas quelantes pueden ser proteínas de fusión de albúmina fijadas del invento para facilitar rotulación de dicha proteína con iones metálicos incluyendo radionúclidos o rótulos fluorescentes. Por ejemplo, ver Subramanian, R y Meares, C.F., "Agentes Quelantes Bifuncionales para Anticuerpos Monoclonales Rotulados por Radiometales," en *Cancer Imaging with Radiolabeled Antibodies* (D.M. Godenberg, Ed.) Kluwer Academic Publications, Boston; Saji, H. "Liberación dirigida de imágenes radiorotuladas y agentes terapéuticos: radiofarmacéuticos bifuncionales." *Crit. Rev. Ther. Drug Carrier Syst. 16:209-244* (1999); Srivastava S.C. y Mease R.C., "Avance en investigación sobre ligandos, núclidos y técnicas para rotular anticuerpos monoclonales." *Int. J. Rad. Appl. Instrum. B 18:589-*603 (1991); y Liu, S. y Edwards, D.S., "Quelantes bifuncionales para radiofarmaceuticos de lantanida terapéuticos." *Bioconjug. Chem. 12:*7-34 (2001). Cualquier quelante en cual se puede unir de manera covalente a dichas proteínas de fusión de albúmina se puede utilizar según el presente invento. El quelante puede comprender además una fracción enlazadora que conecta la fracción quelante a la proteína de fusión de albúmina.

En una realización, las proteínas de fusión de albúmina del invento están unidas a un quelante acíclico tal como ácido dietileno triamina-N,N,N',N";N"'-pentacético (DPTA), análogos de DPTA, y derivados de DPTA. Como ejemplos no limitantes, el quelante puede ser ácido 2-(p-isotiocianatobencil)-6-metildietilentriaminapentacético (1B4M-DPTA, también conocido como MX-DTPA), ácido 2-metil-6-(rho-nitrobencil)-1,4,7-triazaheptano-N,N,N',N",N"'-pentacético (nitro-1B4M-DTPA o nitro-MX-DTPA); ácido 2-(p-isotiocianoatobencil)-ciclohexildietilentriaminapentacético (CHX-DTPA), o ácido N-[2-amino-3-(rho-nitrofenil)propil]-trans-ciclohexano-1,2-diamina-N,N',N"-pentacético (nitro-CHX-A-DTPA).

En otra realización, las proteínas de fusión de albumina del invento están fijadas a un quelante de terpiridina acíclico tal como 6,6"-bis[N,N,N",N"-tetra(carboximetil)amino]metil-4'-(3-amino-4-metoxifenil)-2,2':6',2"-terpiridina (TMT-amina).

En ralizaciones específicas, el quelante macrolítico el cual está fijado a la proteína de fusión de albúmina del invento es ácido 1,4,7,10-tetraazaciclododecano-N,N',N"-N"'-tetraacético (DOTA). En otras realizaciones específicas, DOTA está fijado a la proteína de fusión de albumina del invento a través de una molécula enlazadora. Ejemplos de moleculas enzadoras útiles para conjugar DOTA a un polipéptido son comúnmente conocidas en el estado de la técnica ver, por ejemplo, DeNardo, Clin Cáncer Res. 4(10):2483-90 (1998); Peterson, Bioconjug. Chem. 10(4):553-7 (1999); y Zimmerman, Nucl. Med. Biol. 26(8):943-50 (1999); las cuales están incorporadas en la presente como referencia en su totalidad. Además, las patentes Americanas 5.652.361 y 5.756.065, las cuales divulgan agentes quelantes que se pueden conjugar a anticuerpos, y métodos para preparación y utilización de los mismos, son incorporados en la presente como referencia en su totalidad. No obstante las patentes americanas No. 5.652.361 y 5.756.065 se enfocan en la conjugación de agentes quelantes a anticuerpos, alguien con experiencia en el estado de la técnica podría fácilmente adaptar el método divulgado en la presente para conjugar agentes quelantes a otros polipéptidos.

Los quelantes bifuncionales basados en ligandos macrocíclicos en los cuales la conjugación es a través de un brazo activad, o grupo funcional, fijado al esqueleto de carbono del ligando se pueden emplear como se describe en M. Moi, *J. Amer. chem. soc.* 49:2639 (1989) (2-p-nitrobenzil-1,4,7,10-tetraazaciclododecano-N,N',N"-N"'-tetracético); S. V. Deshpande, *J. Nucl.*

Med. 31:473 (1990); G. Ruser, Bioconju. Chem. 1:345 (1990); C.J. Broan, *J.C.S. chem. Comm. 23:1739* (1990); y C.J. Anderson, *J. Nucl. 36*:850 (1995).

En una realización, un quelante macrolítico, tal como quelantes poliazamacrocíclicos, opcionalmente conteniendo uno o más grupos carboxilo, amino, hidroxamato, fosfonato, o fosfato, están fijados a la proteína de fusión de albúmina del invento. En otra realización, el quelante es un quelante seleccionado del grupo que consta de DOTA, análogos de DOTA, y derivados de DOTA.

En una realización, moléculas quelantes apropiadas que se pueden fijar a la proteína fusión de albúmina del invento incluyen DOXA (ácido 1-oxa-4,7,10-triazaciclododecanotriacético), NOTA (ácido 1,4,7-triazaciclononanetriacético), TETA (ácido 1,4,8,11,tetraazaciclotetradecanetetracético), y THT (4'-(3-amino-4-metoxi-fenil)-6-6"-bis(N',N'-dicarboximetil-N-metilhidra zino)-2-2':6',2"-terpiridina), y análogos y derivados de los mismos. Ver, *ej.,* Ohmono, *J. Med. Chem.* 35: 157-162 (1992), Kung, *J. Nucl. Med. 25:* 326-332 (1984); Jurisson, *chem. rev. 93:*1137-1156 (1993); y Patente Americana No. 5.376.080. Otros quelantes apropiados incluyen agentes quelantes divulgados en la Patente U.S. No. 4.647.447; 4.687.659; 4.885.363; EP-A-71564; WO89/00557; y EP-A-232751.

En otras realizaciones, quelantes de ácido carbocíclico macrocíclico apropiados los cuales se puede utilizar en el presente invento incluyen ácido 1,4,7,10-tetraazaciclododecano-N,N',N"-N'"-tetracético (DOTA); ácido 1,4,8,12-tetraazaciclopentadecano-*N,N',N",N'"*-tetracético (15N4); ácido 1,4,7-triazaciclononano-N,N",N'"-triacético (9N3); ácido 1,5,9-triazaciclododecano-N,N',N"-triacético (12N3); y ácido 6-bromoacetamido-bencil-1,4,8,11-tetraazaciclotetradecano-*N,N",N'",N'"'-tetracético* (BAT).

Un quelante preferido que se puede fijar a la proteína de fusión de albúmina del invento es ácido alfa-(5-isotiocianato-2-metoxifenil)-1,4,7,10-tetraazaciclododecano-1,4,7,10-tetracético, el cual también se conoce como MeO-DOTA-NCS. También se puede utilizar una sal o éster de ácido alfa-(5-isotiocianato-2-metoxifenil)-1,4,7,10-tetraazaciclododecano-1,4,7,10-tetracético.

Las proteínas de fusión de albúmina del invento a las cuales los quelantes tales como aquéllos descritos están covalentemente unidos se pueden rotular (a través del sitio de coordinación del quelante), radionúclidos que son apropiados para propósitos terapéuticos, diagnósticos, o ambos terapéuticos y diagnósticos. Ejemplos de metales apropiados incluyen Ag, At, Au, Bi, Ho, In, Lu, Pb, Pd, Pm, Pr, Rb, Rh, Sc, Sr, Tc, Tl, Y, y Yb. Ejemplos de radionúclidos utilizados para diagnósticos son Fe, Gd, ^{111}In, ^{67}Ga, o ^{68}Ga. En otra realización, el radionúclidos utilizado para propósitos diagnósticos es ^{111}In, o ^{67}Ga. Ejemplos de radionúclidos utilizados para propósitos terapéuticos son ^{166}Ho, ^{165}Dy, ^{90}Y, ^{115m}Ln, ^{52}Fe, o ^{72}Ga. En una realización, el radionúclido utilizado para propósitos diagnósticos es ^{166}Ho o ^{90}Y. Ejemplos de los radionúclidos utilizados para propósitos tanto terapéuticos como diagnósticos incluyen ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{175}Yb, o ^{47}Sc. En una realización, el radionúclido es ^{153}Sm, ^{177}Lu, ^{175}Yb, o ^{159}Gd.

Radionúclidos metálicos preferidos incluyen ^{90}Y, ^{99m}Tc, ^{111}Ln, ^{47}Ga, ^{51}Cr, ^{177m}Sn, ^{67}Cu, ^{167}Tm, ^{97}Ru, ^{188}Re, ^{177}Lu, ^{199}Au, ^{47}Sc, ^{67}Ga, ^{51}Cr, ^{177m}Sn, ^{67}Cu, ^{167}Tm, ^{95}Ru, ^{188}Re, ^{177}Lu, ^{199}Au, ^{203}Pb y ^{141}Ce.

En una realización particular, las proteínas de fusión de albúmina del invento a las cuales los quelantes están covalentemente unidos se pueden rotular con un ion metálico seleccionado del grupo que consta de ^{90}Y, ^{111}In, ^{177}Lu, ^{166}Ho, ^{215}Bi, y ^{225}Ac.

Además, los radionúclidos γ-emisores, tales como ^{99m}Tc, ^{111}In, ^{67}Ga, y ^{169}Yb han sido aprobados o están bajo investigación para creación de imágenes de diagnostico, mientras los emisores-β, tales como ^{67}Cu, ^{111}Ag, ^{186}Re, y ^{90}Y son utiles para aplicaciones en terapia tumoral. También otros radionúclidos útiles incluyen emisores-γ, tales como ^{99m}Tc, ^{111}In, ^{67}Ga, y ^{169}Yb, y

emisores- β, tales como ^{67}Cu, ^{111}Ag, ^{186}Re y ^{90}Y, así como también otros radionúclidos de interés tales como ^{211}At, ^{212}Bi, ^{177}Lu, ^{86}Rb, ^{106}Rh, ^{153}Sm, ^{198}Au, ^{149}Pm, ^{85}Sr, ^{142}Pr, ^{214}Pb, ^{109}Pb, ^{166}Ho, ^{208}TI, y ^{44}Sc. Las proteínas de fusión de albúmina del invento a las cuales los quelantes están covalentemente unidos se pueden rotular con los radionúclidos descritos anteriormente.

En otra realización, las proteínas de fusión de albúmina del invento a las cuales los quelantes están covalentemente unidos se pueden rotular con iones metálicos paramagnéticos incluyendo iones de transición y metal de lantanida, tales como metales con números atómicos de 21-29, 42, 43, 44, o 57-71, en particular iones de Cr, V, Mn, Fe, Co, Ni, Cu, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, y Lu. Los metales paramagnéticos utilizados en composiciones para imágenes de resonancia magnetica incluyen los elementos que tienen números atomicos de 22 a 29, 42, 44 y 58-70.

En otra realización, las proteinas de fusión de albumina del invento a las cuales los quelantes están covalentemente unidos, se pueden rotular con iones metálicos fluorescentes incluyendo lantanidas, en particular La, Ce, Pr, Nd, Pm, Sm, Eu (ej., 152Eu), Gd, Tb, Dy, Ho, Er, Tm, Yb, y Lu.

En otra realización, las proteínas de fusión de albúmina del invento a las cuales los quelantes están covalentemente unidos, se pueden rotular con reporteros que contienen metales pesados los cuales pueden incluir átomos de Mo, Bi, Si, y W.

También es posible rotular las proteínas de fusión de albúmina con un compuesto fluorescente. Cuando el anticuerpo rotulado de manera fluorescente es expuesto a la luz de la longitud de onda adecuada, su presencia se puede entonces detectar debido a la fluorescencia. Entre los compuestos rotuladores fluorescentes más comúnmente utilizados están isocianato de fluoresceina, rodamina, ficoeritrina, ficocianina, aloficocianina y fluorescamina.

Las proteínas de fusión de albúmina también se pueden rotular de manera detectable utilizando metales emisores de fluorescencia tales como 152Eu, u otros de la serie lantanida. Estos metales se pueden fijar al anticuerpo utilizando grupos quelantes metálicos tales como ácido dietilentriaminapentacético (DTPA) o dietilentriaminatetracético (EDTA).

Las proteínas de fusión de albúmina también se pueden rotular de manera detectable al acoplarlas a un compuesto quimioluminiscente. La presencia de una proteína de fusión de albúmina con etiqueta quimioluminiscente luego es determinada al detectar la presencia de luminiscencia que se origina durante el curso de una reacción química. Ejemplos de compuestos rotuladores quimioluminiscentes particularmente útiles son luminol, isoluminol, acridinio-éster teromático, sal de acridinio y éster de oxalato.

Igualmente, un compuesto bioluminiscentes se puede utilizar para rotular proteínas de fusión de albúmina del presente invento. La bioluminiscencia es un tipo de quimioluminiscencia encontrada en sistemas biológicos en los cuales una proteína catalítica aumenta la eficiencia de la reacción quimioluminiscente. La presencia de una proteína bioluminiscente es determinada al detectar la presencia de luminiscencia. Compuestos bioluminiscentes importantes para propósitos de rotulación son luciferina, luciferasa y aequorina.

Organismos transgénicos

Los organismos transgénicos que expresan las proteínas de fusión de albúmina del invento también están incluidos en el invento. Los organismos transgénicos son organismos genéticamente modificados en los cuales material genético recombinante, exógeno o clonado se ha transferido. Tal material genético a menudo es denominado un transgen. La secuencia de ácido nucleicos del transgen puede incluir una o más secuencias reguladoras transcripciones y otras secuencias de aminoácidos tales como intronas, que pueden ser necesarias para expresión óptima y secreción de la proteína codificada. El transgen se puede diseñar para dirigir la expresión de la

proteína codificada en una forma que facilite su recuperación desde el organismo o desde un producto preparado por el organismo, ej., desde leche, sangre, orina, huevos, cabello o semillas del organismo. El transgen puede constar de secuencias de ácido nucleico derivadas del genoma de la misma especie, de una especie diferente que la especie del animal objetivo. El transgen puede ser integrado ya sea en un sitio de un genoma donde esa secuencia de ácidos nucleicos particular no es de otra manera encontrada normalmente o en el sitio normal para el transgen.

El término "organismo transgénico de línea celular germinal" se refiere a un organismo transgénico en el cual la alteración genética o información genética fue introducida en una línea celular germinal, confiriendo así capacidad del organismo transgénico para transferir la información genética a la progenie. Si la progenie en realidad posee alguna o toda esa alteración o información genética, entonces estos también son organismos transgénicos. La alteración o información genética puede ser extraña a la especie de organismo al cual pertenece el receptor, extraña solamente al receptor individual particular, o puede ser información genética ya poseída por el receptor. En el último caso, el gen alterado o introducido se puede expresar de manera diferente que el gen nativo.

Un organismo transgénico puede ser un animal transgénico o una planta transgénica. Los animales transgénicos se pueden producir mediante una variedad de métodos diferentes incluyendo transfección, electroporación, microinyección, direccionamiento génico en células madre enbrionarias e infección viral y retroviral recombinante (*ver., ej.,* Patente Americana No. 4.736.866; Patente Americana No. 5.602.307; Mullins (1993) Hipertensión 22(4):630-633; Brening (1997) Surg.Oncol 6(2)99-110; Tuan (ed.), *Recombinant Gene Expression Protocols,* Métodos en biología molecular No. 62, Humana Press (1997)). El método de introducción de fragmentos de ácido nucleico en células mamíferas competentes de recombinación puede ser mediante cualquier método que favorezca co-transformación de múltiples moléculas de ácido nucleico. Los procedimientos detallados para producir animales transgénicos son facilmente disponibles para alguien con experiencia en el estado de la técnica, incluyendo las divulgaciones en la Patente Americana No. 5.489.743 y Patente Americana 5.602.307.

Un número de ratones transgénicos recombinantes han sido producidos, incluyendo aquellos los cuales expresan una secuencia oncogena codificada (Patente Americana No. 4.736.866); expresan antigeno-T simio SV40 (Patente Americana No. 5.728.915); carecen de la expresión de factor regulador de interferón 1 (IRF-1) (Patente Americana No. 5.731.490); exhiben disfunción dopaminérgica (Patente Americana No. 5.723.719); expresan al menos un gen humano el cual participa en control de presión sanguinea (Patente Americana No. 5.731.489); presentan mayor similitud a las condiciones existentes en la enfermedad de Alzheimer de ocurrencia natural (Patente Americana No. 5.720.936); tienen capacidad reducida para mediar en adhesión celular (Patente Americana No. 5.602.307); poseen un gen de la hormona de crecimiento humano (Clutter (1966) Genetics 143(4):1753-1760); o son capaces de generar una respuesta a anticuerpo completamente humano (McCarthy (1997) The Lancet 349(9049):405).

Mientras ratones y ratas siguen siendo los animales de elección para la mayoría de experimentación transgénica, en algunas instancias es preferible o necesario utilizar especies animales alternativas. Los procedimientos transgénicos se han utilizado exitosamente en una variedad de animales no murinos, incluyendo ovejas, cabras, cerdos, perros, gatos, monos, chimpancés, hamsters, conejos, vacas y conejillos de Indias (*ver,* ej., Kim (1997) Mol. Reprod. Dev. 46(4):515-526; Houdebine (1995) Reprod. Nutr. Dev. 35(6)-609-617; Petters (1994) Reprod. Fertil. Dev. 6(5):643-645; Schnieke (1997) Science 278(5346):2130-2133; y Amoah (1997) J. Animal Science 75(2)-578-585).

Para dirigir la secreción de la proteína codificada por el transgen del invento en la leche de mamíferos transgénicos, se puede colocar bajo el control de un promotor que sea preferencialmente activado en células epiteliales mamíferas. Se prefieren promotores que controlan los genes que codifican proteínas de leche, por ejemplo el promotor para caseina, beta lactoglobulina (ver, ej., DiTullio (1992) BioTechnology 10:74-77; Clark (1989) BioTecnology 7:487-492; Gorton (1987) BioTecnology 5:1183-1187; y Soulier (1992) FEBS Letts. 297:13). Los mamíferos transgénicos de elección producirían grandes cantidades de leche y tendrían largos periodos de lactancia, por ejemplo cabras, vacas, camellos u ovejas.

Una proteína de fusión de albúmina del invento también se puede expresar en una planta transgénica, ej. una planta en la cual el transgen de ADN es insertado en el genoma nuclear o plastídico. Los procedimientos de transformación vegetal utilizados para producir ácidos nucleicos extranjeros en células vegetales o protoplastos son conocidos en el estado la técnica. Ver, en general, Métodos en Enzimologia Vol. 153 ("ADN recombinante Parte D"), Wu y Grossman Eds., Academic Press y Solicitud de Patente Europea EP 693554. Métodos para generación de plantas geneticamente modificadas son adicionalmente descritos en la Patente Americana No. 5.283.184, Patente Americana No. 5.482.852, y Solicitud de Patente Europea EP 693 554, todas estas incorporadas en la presente como referencia.

Composiciones farmacéuticas o terapéuticas

Las proteínas de fusión de albúmina del invento o fórmulaciones de las mismas se pueden administrar mediante cualquier método convencional incluyendo inyección parenteral (ej. subcutanea o intramuscular) o infusión intravenosa. El tratamiento puede constar de una única dosis o una pluralidad de dosis durante un periodo de tiempo.

Mientras es posible que una proteína de fusión de albúmina del invento sea administrada sola, es preferible presentar ésta como una fórmulación farmacéutica, junto con uno o más vehículos aceptables. Los vehículos deben ser "aceptables" en el sentido de ser compatibles con la proteína de fusión de albúmina y no perjudiciales a los receptores de los mismos. Típicamente, los vehículos serán agua o sustancia salina las cuáles serán estériles y libres de pirógenos. Las proteínas de fusión de albúmina del invento particularmente son bien adaptadas a fórmulación en vehículos acuosos tal como sustancia salina o agua estéril libre de pirógenos u otras soluciones isotónicas debido su vida en almacenamiento extendida en solución. Por ejemplo, los composiciones farmacéuticas del invento se pueden fórmular por adelantado en forma acuosa, por ejemplo, semanas o meses o periodos de tiempo más largos antes de ser dispensadas.

Por ejemplo, las fórmulaciones que contienen la proteína de fusión de albúmina se pueden preparar tomando en cuenta el vida en almacenamiento extendida de la proteína de fusión de albúmina en fórmulaciones acuosas. Como se discutió anteriormente, la vida en almacenamiento de muchas de estas proteínas terapéuticas es marcadamente aumentada o prolongada después de fusión con HA.

En instancias donde la administración en aerosol es apropiada, las proteínas de fusión de albúmina del invento se pueden fórmular como aerosoles utilizando procedimientos estándares. El término "aerosol" incluye cualquier fase suspendida proveniente de gas de una proteína de fusión de albúmina del presente invento la cual es capaz de ser inhalada en los bronquiolos o pasajes nasales. Específicamente, aerosol incluye una suspensión proveniente de gas de gotas de una proteína de fusión de albúmina del presente invento, como puede producirse en un nebulizador o inhalador de dosis medidas, o en un atomizador de rocío. Aerosol también incluye una composición de polvo seco de un compuesto del presente invento suspendida en aire u otro gas portador, el cual puede ser liberado mediante insuflación desde un dispositivo inhalador, por ejemplo. Ver Ganderton & Jones, *Drug Delivery to Respiratory Tract, Ellis Horwood* (19 87);

Gonda (1990) *Critical Reviews in Therapeutic Drug Carrier Systems* 6:273-313; y Raeburn (1992) *Pharmacol. Toxicol. Methods* 27:143-159.

Típicamente las fórmulaciones del invento también son no inmunogénicas, en parte, debido al uso de los componentes de la proteína de fusión de albúmina siendo derivada de la especie adecuada. Por ejemplo, para uso humano, tanto la proteína terapéutica como las porciones de albúmina de la proteína de fusión de albúmina típicamente serán humanas. En algunos casos, en donde uno u otro componentes no es humano-derivado, ese componente puede ser humanizado mediante sustitución de aminoácidos claves de manera que epítopes específicos aparecen al sistema inmune humano para ser humanos en naturaleza más que extranjeros.

Las fórmulaciones se pueden presentar convenientemente en forma de dosificación unitaria y se pueden preparar mediante cualquiera de los métodos conocidos en el estado de la técnica de farmacia. Tales métodos incluyen el paso de poner en asociación la proteína de fusión de albúmina con los vehículos que constituyen uno o más ingredientes accesorios. En general las fórmulaciones son preparadas al poner en asociación de manera uniforme e íntima, el ingrediente activo con vehículos líquidos o vehículos sólidos divididos de manera fina o ambos y luego, si es necesario formar el producto.

Fórmulaciones apropiadas para administración parenteral incluyen soluciones de inyección estéril acuosas y no acuosas las cuáles pueden contener antioxidantes, amortiguadores, bacteriostatos y solutos los cuales hacen la fórmulación apropiada para el receptor; y suspensiones estériles acuosas y no acuosas las cuáles pueden incluir agentes de suspensión y agentes espesantes. Las fórmulaciones se pueden presentar en recipientes de dosis unitarias o múltiples dosis, por ejemplo ampollas selladas, recipientes o jeringas, y se pueden almacenar en una condición de secado por congelamiento (liofilizada) requiriendo solamente la adición del vehículo líquido estéril, por ejemplo agua para inyecciones, inmediatamente antes de utilizar. Las soluciones de inyección extemporáneas y suspensiones se pueden preparar a partir de polvos estériles. Fórmulaciones de dosificación pueden contener la porción de proteína terapéutica a una concentración molar baja o dosis baja en comparación con la fórmulación estándar sin fusionar para la proteína terapéutica dada la vida media sérica extendida exhibida por muchas de las proteínas de fusión de albúmina del invento.

Como un ejemplo, cuando la proteína de fusión de albúmina del invento comprende una de las proteínas listadas en la columna "Proteína Terapéutica:X" de la tabla 1 como una o más de las regiones de proteínas Terapéuticas, la forma de dosificación se puede calcular sobre la base de la potencia de la proteína de fusión de albúmina con relación a la potencia de la proteína terapéutica sola, mientras se toma en cuenta la vida media sérica prolongada y la vida en almacenamiento de las proteínas de fusión de albúmina en comparación con proteínas terapéuticas nativas. Por ejemplo, si la proteína terapéutica típicamente es administrada entre 0.3 y 30.0 IU/kg/semana, o 0.9 y 12.0 IU/kg/semana, suministrada en tres o cuatro dosis divididas durante un año o más. En una proteína de fusión de albumina que consta de HA de longitud total fusionada a una proteína terapeutica, una dosis equivalente en términos de unidades representaría un mayor peso de agente pero la frecuencia de dosificación se puede reducir, por ejemplo a dos veces por semana, una vez por semana o menos.

Las fórmulaciones o composiciones del invento se pueden empacar junto con, o incluir en un kit con instrucciones o un inserto de empaque que se refiere a la vida en almacenamiento extendida del componente de proteína de fusión de albúmina. Por ejemplo, tales instrucciones o insertos de empaque pueden direccionar condiciones de almacenamiento recomendadas, tales como tiempo, temperatura y luz, tomando en cuenta la vida en almacenamiento extendida o prolongada de las proteínas de fusión de albúmina del invento. Tales instrucciones o insertos de

empaque también pueden direccionar las ventajas particulares de las proteínas de fusión de albúmina del invento, tal como la facilidad de almacenamiento para fórmulaciones que pueden requerir uso en el campo, fuera de condiciones controladas de hospitales, clínicas o consultorios. Como se describió anteriormente, las fórmulaciones del invento pueden estar enu forma acuosa o pueden estar almacenadas bajo circunstancias menores a las ideales sin pérdida significativa de actividad terapéutica.

Las proteínas de fusión de albúmina del invento también se pueden incluir en nutracéuticos. Por ejemplo, ciertas proteínas de fusión de albúmina del invento se pueden administrar en productos naturales, incluyendo leche o productos lácteos obtenidos de un mamífero transgénico que expresa la proteína de fusión de albúmina. Tales composiciones también incluyen plantas o productos vegetales obtenidos a partir de una planta transgénica la cual expresa la proteína de fusión de albúmina. La proteína de fusión de albúmina también se puede proporcionar en polvo o forma de tableta, con o sin otros aditivos, vehículos, rellenos y diluyentes conocidos. Los nutracéuticos se describen en Scott Hegenhart, *Food Product Design*, Dec. 1993.

En invento también proporciona métodos de tratamiento y/o prevención de enfermedades o trastornos (tales como, por ejemplo, cualquiera o más de las enfermedades o trastornos divulgados en la presente) mediante administración a un sujeto de una cantidad efectiva de una proteína de fusión de albúmina del invento o un polinucleotido que codifica una proteína de fusión de albúmina del invento ("polinucleotido de fusión de albúmina") en un vehículo farmacéuticamente aceptable.

La proteína de fusión de albúmina y/o polinucleotido serán fórmuladas y dosificadas en una forma consistente con buena práctica médica, tomando en cuenta la condición clínica del paciente individual (especialmente los efectos secundarios de tratamiento con la proteína de fusión de albúmina y/o polinucleotido sólo), el sitio de liberación, el método de administración, el esquema de administración, y otros factores conocidos por médicos. La "cantidad efectiva" para propósitos en la presente es determinada así mediante tales consideraciones.

Como una proposición general, la cantidad farmacéuticamente efectiva total de la proteína de fusión de albúmina administrada parenteralmente por dosis estará en el rango entre 1ug/kg/día y 10 mg/kg/día, y más preferiblemente para humanos entre 0.01 y 1 mg/kg/día aproximadamente para la hormona. Si se suministra continuamente, la proteína de fusión de albúmina tipicamente se administra a una tasa de dosis entre 1 ug/kg/hora y 50 ug/kg/hora aproximadamente, ya sea mediante 1-4 inyecciones por día o mediante infusiones subcutaneas continuas, por ejemplo, utilizando una mini-bomba. También se puede utilizar una solución de bolsa intravenosa. La longitud de tratamiento necesario para observar cambios y el intervalo después de tratamiento para que ocurran las respuestas, parece variar dependiendo del efecto deseado.

Como se anotó anteriormente, la proteína fusión de albúmina del invento tiene una estabilidad plasmática superior en comparación con la porción de proteína terapéutica (o fragmento o variante la misma) sola. Este aumento en estabilidad plasmática dede ser tomado en cuenta cuando se determina la cantidad efectiva de la proteína de fusión de albúmina a ser administrada por dosis y el esquema de administración de dosificación. En particular, la mayor estabilidad plasmática puede permitir que la proteína de fusión de albúmina sea administrada a una dosis menor a la misma frecuencia de administración, o alternativamente, puede permitir que la proteína fusión de albúmina sea administrada en menos dosis. Preferiblemente, la mayor estabilidad permite que la proteína de fusión de albúmina del invento sea administrada con menos frecuencia en menos dosis. Preferiblemente, la mayor estabilidad permite que la proteína de fusión de albúmina del invento sea administrada con menos frecuencia en menos dosis. Más

preferiblemente, la proteína de fusión de albúmina del invento se puede administrar una vez cada dos semanas. Aún más preferiblemente, la proteína de fusión de albúmina se puede administrar una vez cada 3, 4,5, o más semanas dependiendo de la fármacocinética de la proteína de fusión de albúmina. Por ejemplo, como se discutió anteriormente, la fármacocinéticas de una proteína de fusión IFN-alfa-HSA soporta un régimen de dosificación de una vez cada 2-4 semanas o más, y aún dosificación a intervalos de cuatro semanas o más de cuatro semanas.

Las proteínas de fusión de albúmina y/o polinucleotidos se pueden administrar de manera oral, rectal, parenteral, intrasternal, intravaginal, intraperitoneal, tópica (mediante polvos, ungüentos, geles, gotas o parches transdermales), bucal, o como un espray oral o nasal. "Vehículo farmacéuticamente aceptable" se refiere a un relleno sólido no tóxico, semisólido o líquido, diluyente, material encapsulante o auxiliar de fórmulación de cualquiera. El término "parenteral" como se utiliza en la presente se refiere a modos de administración los cuales incluyen inyección e infusión intravenosa, intramuscular, intraperitoneal, intracisternal, subcutanea, e intraarticular.

Las proteínas de fusión de albúmina y/o polinucleotidos del invento también son administrados de manera apropiada mediante sistemas de liberación sostenida. Ejemplos de proteínas de fusión de albúmina y/o polinucleotidos de liberación sostenida son administrados de manera oral, rectal, parenteral, intracisternal, intravaginal, intraperitoneal, tópica (mediante polvos, ungüentos, geles, gotas o parches transdermales), bucal, o como un espray oral o nasal. "Vehículo farmacéuticamente aceptable" se refiere a un relleno sólido no tóxico, semisólido o líquido, diluyente, material encapsulante o auxiliar de fórmulación de cualquier tipo. El término "parenteral" como se utiliza en la presente se refiere a modos de administración los cuales incluyen inyección e infusión intravenosa, intramuscular, intraperitoneal, intrasternal, subcutanea, e intraarticular. Ejemplos adicionales de proteínas de fusión de albumina de liberación sostenida y/o polinucleotidos incluyen materiales poliméricos apropiados (tales como, por ejemplo, matrices poliméricas semi-permeables en la forma de artículos formados, ejemplo, películas o microcápsulas), materiales hidrofóbicos apropiados (por ejemplo, como una emulación en un aceite aceptable) o resinas de intercambio iónico, y derivados apenas solubles (tal como, por ejemplo, una sal apenas soluble).

Matrices de liberación sostenida incluyen polilactidos (Patente Americana No. 3.773.919, EP 58.481), copolímeros de ácido L-glutamico y gama-etil-L-glutamato (Sidman, Biopolímeros 22:547-556 (1983)), poly (metacrilato de 2-hidroxietilo) (Langer, J. Biomed. Mater. Res. 15:167-277 (1981), y Langer, Chem. Tech. 12:98-105 (1982)), acetato etilenvinílico (langer, Id) o ácido poly-D-(-)-3-hidroxibutírico (EP 133.988).

Proteínas de fusión de albúmina y/o polinucleotidos de liberación sostenida también incluyen proteínas de fusión de albúmina liposomalmente atrapadas y/o polinucleotidos del invento (*ver* generalmente, Langer, *science* 249:1527-1533 (1990); Treat, *en Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berstein y Fidler (eds.), Liss, New York, pp. 317 - 327 y 353-365 (1989)). Liposomas que contienen la proteína de fusión de albúmina y/o polinucleotido son preparados mediante métodos conocidos *per se*: DE 3.218.121; Epstein, Proc. Natl. Acad. Sci (USA) 82:3688-3692 (1985); Hwang, Proc. Natl. Acad. Sci (USA) 77:4034 (1980); EP 52.322; EP 52.322; EP 36.676; EP 88.046; EP 143.949; EP 142.641; Solicitud de Patente Japonesa 83-118008; Patente Americana No. 4.485.045 y 4.544.545; y EP 102.324. Comúnmente, los liposomas son de tipo unilaminar pequeño (aproximadamente 200-800 Angstroms) en los cuales el contenido lípido es mayor que 30 mol. por ciento de colesterol, la proporción seleccionada es ajustada para optima Terapéutica.

En aún una realización adicional, las proteínas de fusión de albúmina y/o polinucleotidos del invento son liberados mediante una bomba (*ver* Langer, Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald, Surgery 88:507 (1980); Saudek, N. Engl. J. Med. 321:574 (1989)).

Otros sistemas de liberación controlada se discuten en la reseña de Langer (Science 249:1527-1533 (1990)).

Para administración parenteral, en una realización, la proteína de fusión de albúmina y/o polinucleotido es fórmulado generalmente al mezclar este en el grado deseado de pureza, en una forma inyectable de dosificación unitaria (solución, suspensión, o emulsión), con un vehículo farmacéuticamente aceptable, es decir, uno que no sea tóxico para los receptores en las dosificaciones y concentraciones empleadas y que sea compatible con otros ingredientes de la fórmulación. Por ejemplo, la fórmulación preferiblemente no incluye agentes oxidantes y otros compuestos que son conocidos como perjudiciales para el terapéutico.

Generalmente, las fórmulaciones son preparadas al poner en contacto la proteína de fusión de albúmina y/o polinucleotido de manera uniforme e íntima con vehículos líquidos o vehículos sólidos divididos de manera fina o ambos. Luego, si es necesario, el producto es formado en la fórmulación deseada. Preferiblemente el vehículo es un vehículo parenteral, más preferiblemente una solución isotónica con la sangre del receptor. Ejemplos de tales vehículos portadores incluyen agua, solución salina, solución de Ringer, y solución de dextrosa. Vehículos no acuosos tales como aceites fijos y oleato de etilo también son útiles en la presente, así como también los liposomas.

El vehículo apropiadamente contiene cantidades menores de aditivos tales como sustancias que aumentan la isotonicidad y estabilidad química. Tales materiales no son tóxicos para los receptores en las dosificaciones y concentraciones empleadas, e incluyen amortiguadores tales como fosfato, citrato, succinato, ácido acético, y otros ácidos orgánicos o sus sales; antioxidantes tales como ácido ascórbico; polipéptidos de bajo peso molecular (menor que aproximadamente 10 residuos), ej., poliarginina o tripéptidos; proteínas, tales como albúmina sérica, gelatina, o inmunoglobulinas; polímeros hidrofóbicos tales como polivinilpirrolidona; aminoácidos, tales como glicina, ácido glutámico, ácido aspártico, o arginina; monosacaridos, disacaridos, y otros carbohidratos incluyendo celulosa o sus derivados, glucosa, manosa, o dextrinas; agentes quelantes tales como EDTA; alcoholes de azucar tales como manitol o sorbitol; contraiones tales como sodio; y/o surfactantes noniónicos tales como polisorbatos (incluyendo, por ejemplo, Tween-20), poloxameros, o PEG.

La proteína de fusión de albúmina típicamente se fórmula en tales vehículos a una concentración entre 0.1 mg/ml y 100 mg/ml aproximadamente, preferiblemente 1-10 mg/ml, a un pH entre aproximadamente 3 y 8. Se entenderá que el uso de ciertos excipientes extranjeros, vehículos o estabilizantes resultará en la formación de sales polipeptídicas.

Cualquier farmacéutico utilizado para administración terapéutica puede ser estéril. La esterilidad es lograda fácilmente mediante filtración a través de membranas de filtración estériles (ejemplo, membranas de 0.2 micras). Las proteínas de fusión de albúmina y/o polinucleotidos generalmente son colocados en un recipiente con un puerto de acceso estéril, por ejemplo, una bolsa o recipiente de solución intravenosa con un tope perforable mediante una aguja de inyección hipodérmica.

Las proteínas de fusión de albúmina comúnmente serán almacenados en recipientes unitarios o de múltiples dosis, por ejemplo, ampollas o recipientes sellados, como una solución acuosa o como una fórmulación liofilizada para reconstitución. Como ejemplo de una fórmulación liofilizada, recipientes de 10 milímetros son llenados con 5 ml de solución acuosa estéril-filtrada 1%(w/v) de proteína de fusión de albúmina y/o polinucleotido, y la mezcla

resultante es liofilizada. La solución de infusión es preparada mediante reconstitución de la proteína de fusión de albúmina y/o polinucleotido liofilizada utilizando agua para inyección bacteriostatica.

En una realización específica y preferida, las fórmulaciones de proteína de fusión de albúmina comprenden 0.01 M fosfato de sodio, 0.15 mM cloruro de sodio, 0.16 micromoles de octanoato sódico/miligramo de proteína de fusión, 15 ug/mililitro de polisorbato 80, pH 7.2. En otra realización específica y preferida, las fórmulaciones de proteína de fusión de albúmina constan de 0.01 M fosfato de sodio, 0.15 mM cloruro de sodio, 0.16 uM octanoato de sodio/miligramo de proteína de fusión, 15 microgramos/mililitro de polisorbato 80, 7.2. El pH y el amortiguador son eligidos para corresponder a condiciones fisiológicas y la sal se agrega como un tonificador. Se ha elegido octanoato de sodio debido su capacidad reportada para aumentar la estabilidad normal de la proteína en solución. Finalmente, se ha agregado polisorbato como un surfactante genérico, el cual reduce la tensión de superficie de la solución y reduce la absorción no específica de la proteína de fusión de albúmina al sistema de cierre del recipiente.

El invento también se proporciona un kit o paquete farmacéutico que comprende uno más recipientes llenos con uno más de los ingredientes de las proteínas de fusión de albúmina y/o polinucleotidos del invento. Asociada con tales recipientes puede haber una nota en la forma prescrita por una agencia gubernamental que regula la fabricación, uso o venta de farmacéuticos o productos biológicos, la nota refleja la aprobación de la agencia para la fabricación, uso o venta para administración humana. Además, las proteínas de fusión de albúmina y/o polinucleotidos se pueden emplear en conjunción con otros compuestos terapéuticos.

Las proteínas de fusión de albúmina y/o polinucleotidos del invento se pueden administrar solos o de combinación con coadyuvantes. Los coadyuvantes que se pueden administrar con las proteínas de fusión de albúmina y/o polinucleotidos del invento incluyen, pero no están limitados a alumbre, alumbre más desoxicolato (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG (ej., THERACYS®), MPL y preparaciones no viables de *Corynebacterium parvum*. En una realización específica, las proteínas de fusión de albúmina y/o polinucleotidos del invento se administran en combinación con alumbre. En otra realización específica, las proteínas de fusión de albúmina y/o polinucleotidos del invento se administran en combinación con QS-21. Coadyuvantes adicionales que se pueden administrar con las proteínas de fusión de albúmina y/o polinucleotidos del invento incluyen, pero no están limitados a inmunomodulador lípido de monofosforilo, AdjuVax 100a, QS-21, QS-18, CRL1005, sales de aluminio, MF-59, y tecnología de coadyuvante virosomal. Las vacunas que se pueden administrar con las proteínas de fusión de albúmina y/o polinucleotidos del invento, incluyen pero no están limitados a vacunas dirigidas a protección contra MMR (sarampión, paperas, rubéola), polio, varicela, tétanos/difteria, hepatitis A, hepatitis B, *Haemophilus influenzae* B, tos ferina, neumonía, influenza, enfermedad de lyme, rotavirus, cólera, fiebre amarilla, encefalitis japonesa, poliomielitis, hidrofobia, y fiebre tifoidea. Se pueden administrar combinaciones ya sea de manera concomitante, ej., como una mezcla, de manera separada pero simultánea o de manera concurrente; o secuencialmente. Esto incluye presentaciones en las cuales los agentes combinados son administrados juntos como una mezcla terapéutica, y también procedimientos en los cuales los agentes combinados son administrados de manera separada pero simultánea, ej., a través de líneas intravenosas separadas hacia el mismo individuo. Administración "en combinación" incluye además la administración separada de uno de los compuestos o agentes suministrado primero, seguido por el segundo.

Las proteínas de fusión de albúmina y/o polinucleotidos del invento se pueden administrar solos o en combinación con agentes terapéuticos. La proteína fusión de albúmina y/o agentes nucleotidos que se pueden administrar en combinación con la proteína fusión de albúmina y/o

polinucleotidos del invento, incluyen pero no están limitados a agentes quimioterapéuticos, antibióticos, antiinflamatorios esteroidales y no esteroidales, agentes inmunoterapéuticos convencionales, y/o tratamientos terapéuticos descritos más adelante. Se pueden administrar combinaciones ya sea concomitantemente, ej., como una mezcla, de manera separada pero simultánea o concurrentemente; o secuencialmente. Esto incluye presentaciones en las cuales los agentes combinados son administrados juntos como una mezcla terapéutica, y también procedimientos en los cuales los agentes combinados son administrados de manera separada pero simultánea, ej., a través de líneas intravenosas separadas hacia el mismo individuo. Administración "en combinación" además incluye administración separada de uno de los compuestos o agentes suministrado primero, seguido por el segundo.

En una realización, las proteínas de fusión de albúmina y/o polinucleotidos del invento son administrados en combinación con un anticoagulante. Los anticoagulantes que se pueden administrar con las composiciones del invento incluyen, pero no están limitados a heparina, heparina de bajo peso molecular, warfarina sódica (ej., COUMADIN®), dicumarol, 4-hidoxicoumarina, anisindiona (ej., MIRADON™), acenocoumarol (ej., nicoumalone, SINTHROME™), indan-1,3-diona, fenprocoumon (ej., MARCUMAR™), biscoumacetato de etilo (ej., TROMEXAN™), y aspirina. En una realización específica, los compuestos del invento son administrados en combinación con heparina y/o warfarina. En otra realización específica, las composiciones del invento son administrados en combinación con warfarina y aspirina. En otra realización específica, las composiciones del invento son administradas en combinación con heparina. En otra realización específica, las composiciones del invento son administrados en combinación con heparina y aspirina.

En otra realización, las proteínas de fusión de albúmina y/o polinucleotidos del invento son administrados en combinación con fármacos trombolíticos. Los fármacos trombolíticos que se pueden administrar con las composiciones del invento incluyen pero no están limitados a plasminógeno, lys-plasminógeno, alfa2-antiplasmin, estreptoquinasa (ej., KABIKINASE™), *antiresplace* (ej., EMINASE™), activador de plasminógeno tisular (T-pa, altevasa, ACTIVASE™), uroquinasa (ej., ABBOKINASE™), sauruplasa, (Prouroquinasa, uroquinasa de cadena simple), y ácido aminocaproico (ej., AMICAR). En una realización específica, las composiciones del invento son administradas en combinación con activador de plasminógeno de tejidos y aspirina.

En otra realización, las proteínas de fusión de albúmina y/o polinucleotidos del invento son administrados en combinación con fármacos antiplaquetas. Los fármacos antiplaquetas que se pueden administrar con las composiciones del invento incluyen, pero no están limitados a, aspirina, dipiridamol (ej., PERSANTINA), ticlopidina (ej., TICLID).

En realizaciones específicas, el uso de anticoagulantes, fármacos trombolíticos y/o antiplaquetas en combinación con proteínas de fusión de albúmina y/o polinucleotidos del invento, es contemplado para prevención, diagnosis, y/o tratamiento de trombosis, trombosis arterial, trombosis venosa, tromboembolismo, embolismo pulmonar, ateroesclerosis, infarto del miocardio, ataque isquémico temporal, angina inestable. En realizaciones específicas, el uso de anticoagulantes, fármacos trombolíticos y/o fármacos antiplaquetas en combinación con proteínas de fusión de albúmina y/o polinucleotidos del invento, es contemplado para prevención de oclusión de injertos safenos, para reducir el riesgo de trombosis periprocedimiento como el que pudiera acompañar procedimientos de angioplastia, para reducir el riesgo de apoplejía en pacientes con fibrilación atrial incluyendo fibrilación atrial no reumática, para reducir el riesgo de embolismo asociadocon válvulas cardiacas mecánicas y/o enfermedad de válvulas mitrales. Otros

usos para los terapéuticos del invento, solos o en combinación con fármacos antiplaquetas, anticoagulantes, y/o trombolíticos, incluyen, pero no están limitados a la prevención de oclusiones en dispositivos extracorpóreos (ej., canulas intravasculares, derivaciones de acceso vascular en pacientes de hemodialisis, máquinas para hemodialisis y máquinas para derivación cardiopulmonar).

En ciertas realizaciones, las proteínas de fusión de albúmina y/o polinucleotidos del invento se administran en combinación con agentes antiretrovirales, inhibidores nucleosidos/nucleótidos de la transcriptasa inversa (NTTIs *por su sigla en inglés*), inhibidores nucleosidos de la transcriptasa inversa no nucleosida (NNRTIs), y/o inhibidores de proteasa (PIs). Los NRTIs que se pueden administrar en combinación con proteínas de fusión de albúmina y/o polinucleotidos del invento, incluyen, pero no están limitados a RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosina/ddI), HIVID™ (zalcitabine(ddC), ZERIT™ (estavudina(d4T), EPIVIR™ (lamivudina/3TC), y COMBIVIR™ (zidovudina). Los NNRTIs que se pueden administrar en combinación con las proteínas de fusión de albúmina y/o polinucleotidos del invento, incluyen, pero no están limitados a VIRAMUNE™ (nevirapina), RESCRIPTOR™ (delavirdina), y SUSTIVA™ (efavirenz). Los inhibidores de proteasa que se pueden administrar en combinación con las proteínas de fusión de albúmina y/o polinucleotidos del invento, incluyen, pero no están limitados a CRIXIVAN™ (indinavir), NORVIN™ (ritonavir), INVIRASE™ (saquinavir), y VIRACEPT™ (nelfinavir). En una realización específica, agentes antiretrovirales, inhibidores nucleosidos de la transcriptasa inversa, inhibidores no nucleosidos de la transcriptasa inversa, y/o inhibidores de la proteasa se pueden utilizar en combinación con proteínas de fusión de albúmina y/o polinucleotidos del invento para tratar SIDA y/o prevenir o tratar infección por VIH.

NRTIs adicionales incluyen LODENOSINE™ (F-ddA; un NRTI de adenosina ácido-estable; Triangle/Abbot; COVIRACIL (emtricitabina/FTC; estrucutalmente relacionada con lamivudina (3TC) pero con 3 a 10 veces más de actividad *in vitro*; Triangle/Abbot); dOTC (BCH-10652, también estrechamente relacionado con lamivudina pero retiene actividad contra una proporción sustancial de aislados resistentes a lamivudina; Biochem Pharma); **Adefovir** (aprobación rechazada para terapia anti-VIH por la FDA; Gilead Sciences); PREVEON® (Adenovir Dipivoxil, el profármaco activo de adefovir; su forma activa es PMEA-pp); **TENOFOVIR™** (bis-POC PMPA, un profármaco PMPA; Gilead); **DAPD/DXG** (metabolito activo de DAPD; Triangle/Abbott); **D-D4FC** (relacionado con 3TC, con actividad contra virus AZT/3TC-resistente); **GW420867X** (Glaxo Wellcome); ZIAGEN (abacavir/159U89; Glaxo Wellcome Inc.); CS-87 (3'-azido-2'-3'-dideoxiuridina; WO 99/66936); y formas del profármaco portador de S-acil-2-tioetil (SATE) de beta-L-FD4C y beta-L-FddC (WO 98/17281).

NNRTIs adicionales incluyen COACTINON™ (Emivirina/MKC-442, NNRTI potente de la clase HEPT; Triangle/Abbott); **CAPRAVIRINA™** (ag-1549/s-1153, un NNRTI de siguiente generación con actividad contra virus que contienen la mutación K103N, Agouron); PNU-142721 (tiene actividad 20 a 50 veces mayor que su precedesor delavirdina y es activo contra mutantes K103N; Pharmacia & Upjohn); DPC-961 y DPC-963 (derivados de segunda geneación de efavirentz, diseñados para ser activos con virus con la mutación K103N; DoPont); GW-420867X (tiene 25 veces más actividad que HBY097 y es activo contra mutantes K103N; Glaxo Wellcome); CALANOLIDE A (un agente de ocurrencia natural proveniente del arból de latex; activo contra virus que contienen las mutaciones Y181C y K103N); y Propolis (WO 99/49830).

Inhibidores de la proteasa adicionales incluyen **LOPINAVIR™** (ABT378/r; Abbot Laboratories); BMS-232632 (un azapéptido; Bristol-Myres Squibb); TIPRANAVIR (PNU-

140690, una dihidropirona no péptida; Pharmacia & Upjohn); PD-178390 (una dihidropirona no péptidica; Parke-Davis); BMS 232632 (un azapeptido; Bristol-Myers Squibb); L-756.423 (un análogo de indinavir; Merck); DMP-450 (un compuesto de urea cíclico; Avid & DuPont); AG-1776 (un peptidomimético con actividad *in vitro* contra virus resistentes a inhibidores de proteasa; Agouron); **VX-175/GW-433908** (fosfato profármaco de amprenavir; Vertex & Glaxo Welcome); **CGP61755** (Ciba); y AGENERASA (amprenavir; Glaxo Wellcome Inc.).

Agentes antiretrovirales adicionales incluyen inhibidores de fusión/aglomerantes gp41. Los inhibidores de fusión/aglomerantes gp41 incluyen T-20 (un péptido de residuos 643-678 del ectodominio de proteína de transmembrana pg41 del VIH el cual se fija a gp41 en su estado de reposo y evita la transformación al estado fusogénico; Trimeris) y T-1249 (un inhibidor de fusión de segunda generación; Trimeris).

Agentes antiretrovirales adicionales incluyen inhibidores de fusión /antagonistas de receptores de quimioquina. Los inhibidores de fusión /antagonistas de receptores de quimioquina incluyen antagonistas CXCR4 tales como AMD 3100 (a biciclam); SDF-1 y sus análogos, y ALX40-4C (un péptido catiónico), T22 (un peptido de 18 aminoácidos; Trimeris) y los T22 análogos T134 y T140; antagonistas CCR5 tales como RANTES (9-68), AOP-RANTES, NNY-RANTES, y TAK-779; y antagonistas CCR5/CXCR4 tales como NSC 651016 (un análogo de distamicina): También incluidos están los antagonistas CCR2B, CCR3, y CCR6. Los agonistas de receptores de quimioquina tales como RANTES, SDF-1, MIP-1alfa, MIP-1beta, etc, también pueden inhibir fusión.

Agentes antiretrovirales adicionales incluyen inhibidores de integrasa. Los inhibidores de integrasa incluyen ácidos dicaffeoilquínicos (DFQA); ácido L-quicórico (un ácido dicafeoiltartárico (DCTA); quinalizarina (QLC) y antraquinonas relacionadas; **ZINTEVIR™** (AR 177, un oligonucleotido que probablemente actua en la superficie celular en cambio de ser un inhibidor de integrasa verdadero; Arondex); y naftoles tales como aquellos divulgados en WO 98/50347.

Agentes antiretrovirales adicionales incluyen compuestos tipo hidroxiurea tales como BCX-34 (un inhibidor de la purina nucleosido fosforilasa; Biocryst); inhibidores de la ribonucleótido reductasa tales como DIDOX (Moleculas para la Salud); inosina monofosfato deshidrogenasa (IMPDH) tales como VX-497 (Vertex); y ácidos micofólicos tales como CellCept (mofetil micofenonato, Roche).

Agentes antiretrovirales adicionales incluyen inhibidores de integrasa viral, inhibidores de traslocación nuclear de genoma viral tales como compuestos de arileno bis(metilcetona); inhibidores de entrada de VIH tales como AOP-RANTES, NNY-RANTES, proteína de fusión RANTES-IgG, complejos solubles de RANTES y glicosaminoglicanos (GAG), y AMD-3100; inhibidores de los dedos de zinc de la nucleocápside tales como compuestos de ditiano; objetivos de VIH Tat y Rev; y farmacoaumentadores tales como ABT-378.

Otras terapias antiretrovirales y terapias adjuntas incluyen citoquinas y linfoquinas tales como MIP-1alfa, MIP-1beta, SDF-1alfa, IL-2, PROLEUKIN (aldesleucina/L2-7001; Chiron), IL-4, IL-10, y IL-13; interferones tales como IFN-alfa2a, IFN-alfa2b, o IFN-beta; antagonistas de TNFs, NFkB, GM-CSF, M-CSF, y IL-10; agentes que modulan activación inmune tal como ciclosporina y prednisona; vacunas tales como Remune (Inmunogen de VIH), APL 400-003 (Apollon), gp120 recombinante y fragmentos, glicoproteína de sobre recombinante bivalente (B/E), rgp.120CM235, MN rgp 120, SF-2 rgp120, complejo gp120/CD4 soluble; proteína Delta JR-FL, péptido sintético ramificado derivado de dominio discontinuo pg120 C3/C4, inmunógenos de fusion competente, y vacunas Gag, Pol, Nef, y Tat; terapías basadas en genes tales como elementos supresores genéticos (GSEs; WO 98/54366); e intraquinas (quimioquinas

CC geneticamente modificadas dirigidas al ER para bloquear expresión de superficie de CCR5 recien sintetizado (Yang, *PNAS* 94:11567-72 (1997); Chen, *Nat. Med.* 3:1110-16 (1997)); anticuerpos tal como el anticuerpo anti-CXCR4 12G5, los anticuerpos anti-CCR5 2D7, 7C7, PA8, PA9, PA10,G PA11, PA12, y PA14, los anticuerpos anti-CD4 Q4120 y RPA-T4, el anticuerpo anti-CCR3 7B11, los anticuerpos anti-gp120 17b, 48d, 447-52D, 257-D, 268-D y 50.1, anticuerpos anti-Tat, anticuerpos anti-TNF-alfa, y anticuerpo monoclonal 33A; agonistas y antagonistas de receptores de hidrocarburo de arilo (AH) tales como TCDD, 3,3',4,4',5-pentaclorobifenil, 3,3', 4,4'-tetraclorobifenil, y alfa-naftoflavona (WO 98/30213); y antioxidantes tales como éster etílico de γ-L-glutamil-L-cisteina (γ-GCE; WO 99/56764).

En una realización adicional, las proteinas de fusión de albúmina y/o nucleotidos del invento son administrados en combinación con un agente antiviral. Agentes antivirales que pueden ser administrados con proteínas de fusión de albúmina y/o polinucleotidos del invento incluyen, pero no están limitados a aciclovir, ribavirina, amantadina, remantidina, maxamina, o timalfasina. Específicamente, la proteína de fusión de albúmina e interferón se puede administrar en combinación con cualquiera de estos agentes. Además, la proteína de fusión de albúmina e interferón alfa también se pueden administrar con cualquiera de estos agentes, y preferiblemente, la proteína de fusión de albúmina e interferón alfa 2a y 2b se puede administrar con cualquiera de estos agentes. Además, la proteína de fusión de albúmina e interferón beta también se pueden administrar con cualquiera de estos agentes.. Adicionalmente, cualquiera de las proteínas de fusión de albúmina híbridas de IFN se puede administrar en combinación con cualquiera de estos agentes.

En una realización más preferida, la proteína de fusión de albúmina e interferón es administrada en combinación con ribavirina. En una realización preferida adicional, la proteína de fusión de albúmina e interferón alfa 2a se administran en combinación con ribavirina. En una realización preferida adicional, la proteína de fusión de albúmina e interferón alfa 2b se administran en combinación con ribavirina. En una realización preferida adicional, la proteína de fusión de albúmina e interferón beta se administran en combinación con ribavirina. En una realización preferida adicional, la proteína de fusión de albúmina e interferón híbrido se administran en combinación con ribavirina.

En otras realizaciones, las proteínas de fusión de albúmina y/o polinucleotidos del invento se pueden administrar en combinación con agentes infecciosos anti-oportunistas. Los agentes anti-oportunistas que se pueden administrar en combinación con las proteínas de fusión de albúmina y/o polinucleotidos del invento, incluyen, pero no están limitados a TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITROMYCIN™,GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRYMETHAMINE™, LEUVOVORIN™, NEUPOGEN™ (FILGRASTIM™/g-csf), Y LEUKINE™ (sargramostatim/GM-CSF). En una realización específica, las proteínas de fusión de albúmina y/o polinucleotidos del invento se utilizan en cualquier combinación con TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINA™, y/o ATOVAQUONE™ para tratar o prevenir de manera profilactica una infección de pneumonia oportunista por *Pneumocistis carinni*. En otra realización específica, las proteínas de fusión de albúmina y/o polinucleotidos del invento se utilizan en cualquier combinación con ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, y/o ETHAMBUTOL™ para tratar o prevenir de manera profiláctica una infección compleja

oportunista por *Mycobacterium avium*. En otra realización específica, proteínas de fusión de albúmina y/o polinucleotidos del invento son utilizados en cualquier combinación con RIFABUTIN™, CLARITHROMYCIN™, y/o AZITROMICIN™ para tratar o prevenir de manera profiláctica una infección oportunista por *Mycobacterium tuberculosis*. En otra realización específica, las proteínas de fusión de albúmina y/o polinucleotidos del invento se utilizan en cualquier combinación con GANCICLOVIR™, FOSCARNET™, y/o CIDOFOVIR™ para tratar o prevenir de manera profiláctica una infección oportunista por citomegalovirus. En otra realización específica, las proteínas de fusión de albúmina y/o polinucleotidos del invento se utilizan en cualquier combinación con FLUCONAZOLE™, ITRACONAZOLE™, y/o KETOCONAZOLE™ para tratar o prevenir de manera profiláctica una infección oportunista micótica. En otra realización específica, las proteínas de fusión de albúmina y/o polinucleotidos del invento se utilizan en cualquier combinación con ACYCLOVIR™ y/o FAMCICLOVIR™ para tratar o prevenir de manera profiláctica una infección oportunista de virus del herpes simplex tipo 1 y/o tipo II. En otra realización específica, las proteínas de fusión de albúmina y/o polinucleotidos del invento se utilizan en cualquier combinación con PYRIMETHAMINA™ y/o LEUCOVORINA™ para tratar o prevenir de manera profiláctica una infección oportunista de *toxoplasma gondii*. En otra realización específica, las proteínas de fusión de albúmina y/o polinucleotidos del invento se utilizan en cualquier combinación con LEUCOVORIN™ y/o NEUPOGEN™ para tratar o prevenir de manera profiláctica una infección bacteriana oportunista.

En una realización adicional, las proteínas de fusión de albúmina y/o polinucleotidos del invento se administran en combinación con un agente antibiótico. Los agentes antibióticos que pueden ser administrados con proteínas de fusión de albúmina y/o polinucleotidos del invento incluyen, pero no están limitados a amoxicilina, beta-lactamasas, aminoglicosidos, beta-lactam (glicopéptido), beta-lactamasas, Clindamicina, cloranfenicol, cefalosporinas, cirprofloxacina, eritromicina, fluoroquinolonas, macrolidos, metronidazol, penicilinas, quinolonas, rapamicina, rifampina, estreptomicina, sulfonamida, tetraciclinas, trimetropim, trimetropim sulfametoxazol, y vancomicina.

En otras realizaciones, las proteínas de fusión de albúmina y/o polinucleotidos del invento son administrados en combinación con inmunoestimulantes. Los inmunoestimulantes que se pueden administrar en combinación con las proteínas de fusión de albúmina y/o polinucleotidos del invento incluyen, pero no están limitados a levamisol (ej., ERGAMISOL™), isoprinosina (ej., INOSIPLEX™), interferones (ej., interferón alfa), e interleucinas (ej., IL-2).

En otras realizaciones, las proteínas de fusión de albúmina y/o polinucleotidos del invento son administrados en combinación con agentes inmunosupresivos. Los agentes inmunosupresivos que se pueden administrar en combinación con las proteínas de fusión de albúmina y/o polinucleotidos del invento incluyen, pero no están limitados a esteroides, ciclosporina, análogos de ciclosporina, metilprednisona de ciclofosfamida, prednisona, azatiprina, FK-506, 15-desoxiespergualina, y otros agentes inmunosupresivos que actúan suprimiendo la función de células T respondedoras. Otros agentes inmunosupresivos que se puede administrar en combinación con proteínas de fusión de albúmina y/o polinucleotidos del presente invento incluyen, pero no están limitados a prednisolona, metotrexato, talidomida, metoxsalén, rapamicina, leflunomida, mizoribina (BREDININ™), brequinar, desoxiespergualina, y azaspirano (SKF 105685), ORTHOCLONE OKT™ 3 (muromonab-CD3), SANDIMMUNE™, NEORAL™, SANGDYA™ (ciclosporina), PROGRAF™ (FK506, tacrolimus), CELLCEPT™ (micofenolato motefil, del cual el metabilito activo es ácido micofenólico), IMURANT™ (azatioprine), glucocorticosteroides, esteroides adrenocorticales tales como DELTASONE™ (prednisone) y

HYDELTRASOL™ (prednisolona), FOLEX™ y MEXATE™ (metotrexato), OXSORALEN-ULTRA™ (metoxsalen) y RAPAMUNE™ (sirolimus). En una realización específica, se pueden utilizar inmunosupresores para prevenir rechazo de trasplante de órganos o médula ósea. En una realización adicional, las proteínas de fusión de albúmina y/o polinucleotidos del invento son administrados solos o en combinación con una o más preparaciones de globulina inmune intravenosa. Las preparaciones de globulina inmune intravenosa que se puede administrar con las proteínas de fusión de albúmina y/o polinucleotidos del invento incluyen, pero no están limitados a GAMMAR™, IVVEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, ATGAM™ (globulina antitimocito), y GAMIMUNE™. En una realización específica, las proteínas de fusión de albumina y/o polinucleotidos del invento se administran en combinación con preparaciones de globulina inmune intravenosa en terapia de transplante (ej., transplante de médula ósea).

En otra realización, las proteínas de fusión de albúmina y/o polinucleotidos del invento se administran sólos o como parte de una terapia de combinación, ya sea in vivo a pacientes o in vitro a células, para tratamiento de cáncer. En una realización específica, las proteínas de fusión de albúmina, particularmente fusiones de IL-2-albúmina, se administran de manera repetida durante inmunoterapia pasiva para cáncer, tal como terapia de transferencia celular adoptiva para melanoma metastático como se describe en Dudley (Science Express, 19 de Septiembre de 2002., en www.scienceexpress.org, incorporada en la presente como referencia en su totalidad).

En ciertas realizaciones, las proteínas de fusión de albúmina y/o polinucleotidos del invento se administran solos en combinación con un agente antiinflamatorio. Los agentes antiinflamatorios que se pueden administrar con proteínas de fusión de albúmina y/o polinucleotidos del invento incluyen, pero no están limitados a corticosteroides (ej., betametasona, budesonida, cortisona, daxametasona, hidrocortisona, metilprednisolona, prednisolona, prednisona, y triamcinolona), fármacos anti-inflamatorios no esteroidales (ej., diclofenaco, diflunisal, etodolac, fenoprofen, floctafenina, flurbiprofeno, ibuprofeno, indometacina, cetoprofeno, meclofenamato, ácido mefenámico, meloxicam, nabumetona, naproxeno, oxaproxina, fenilbutazona, piroxicam, sulindac, tenoxicam, ácido tiaprofénico, y tolmetin.), así como también antihistaminas, derivados de ácido aminoarilcarboxílico, derivados de ácido arilacético, derivados de ácido arilbutírico, ácidos arilcarboxílicos, derivados de ácido arilpropiónico, pirazolas, pirazolonas, derivados de ácido salicílico, tiazinacarboxamidas, ácido e-acetamidocaproico, S-adenosilmetionina, ácido 3-amino-4-hidroxibutírico, amixetrina, bendazac, benzidamina, bucoloma, difenpiramida, ditazol, emorfazona, guaiazuleno, nabumetona, nimesulida, orgoteina, oxaceprol, paranilina, perisoxal, piroxima, proquazona, proxazol, y tenidap.

En una realiación adicional, las composiciones del invento son administrados solas o en combinación con un agente anti-angiogénico. Agentes anti-angiogénicos que se puede administrar con las composiciones del invento incluyen, pero no están limitados a Angiostatina (Entremed, Rockville, MD), Tropopin-1 (Boston Life Sciences, Boston, MA), Factor anti-invasivo, ácido retinoico y derivados del mismo, paclitaxel (Taxol), Suramin, Inhibidor de Tejido de Metaloproteinasa-1, Inhibidor de Tejidos de Metaloproteinasa-2, VEGI, inhibidor-1 de activador de plasminógeno, inhibidor-2 de activador de plasminógeno, y varias formas de los metales de transición más livianos del "grupo d".

Los metales de transición del "grupo d" más livianos incluyen, por ejemplo, vanadio, molibdeno, tungsteno, titanio, niobio, y especies de tantalo. Tales especies metálicas de transición pueden formar complejos metálicos de transición. Complejos apropiados de las especies metalicas de transición antes mencionadas incluyen complejos metalicos de transición oxo.

Ejemplos representativos de complejos de vanadilo incluyen complejos de oxo vanadio tales como complejos de vanadato y vanadilo. Complejos apropiados de vanadato incluyen metavanadato y ortovanadato tales como, por ejemplo, metavanadato de amonio, metavanadato de sodio, y ortovanadato de sodio. Complejos de vanadilo apropiados incluyen, por ejemplo, acetilacetonato de vanadilo y sulfato de vanadilo incluyendo hidratos de sulfato de vanadilo tales como mono y trihidratos de sulfato de vanadilo.

Ejemplos representativos de complejos de tungsteno y molibdeno también incluyen complejos oxo. Complejos de oxo tungsteno apropiados incluyen complejos de óxido de tungstato y tungsteno. Complejos de tungstato apropiados incluyen tungstato de amonio, tungstato de calcio, dihidrato de tungstato de sodio, y ácido tungstico. Oxidos de tungsteno apropiados incluyen óxido de tungsteno (IV) y óxido de tungsteno (VI). Complejos de oxo molibdeno apropiados incluyen molibdato, óxido de molibdeno, y complejos de molibdenilo. Complejos de molibdato apropiados incluyen molibdato de amonio y sus hidratos, molibdato de sodio y sus hidratos, y molibdato de potasio y sus hidratos. Óxidos molibdeno apropiados incluyen óxido de molibdeno (VI), óxido de molibdeno (VI), y ácido molíbdico. Complejos de molibdenilo apropiados incluyen, por ejemplo, acetilacetonato de molibdenilo. Otros complejos apropiados de tungsteno y molibdeno incluyen derivados hidroxo derivados de, por ejemplo, glicerol, ácido tartárico, y azúcares.

Una amplia variedad de otros factores anti-angiogénicos también se puede utilizar dentro del contexto del presente invento. Ejemplos representativos incluyen, pero no están limitados a factor plaquetario 4; sulfato de protamina, derivados de quitina sulfatada (preparados a partir de cascarones de jaiba reina), (Murata, Cancer Res. 51:22-26, (1991)); Complejo Peptidoglican Polisacarido Sulfatado (SP-PG) (la función de este compuesto se puede aumentar mediante la presencia de esteroides tales como estrogeno, y citrato de tamoxifen); Estaurosporina; moduladores de metabolismo de matriz, incluyendo por ejemplo, análogos de prolina, cishidroxiprolina, d,L-3,4-dehidroprolina, Tiaprolina, alfa,alfa-dipiridilo, fumarato de aminopropionitrilo; 4-propil-5-(4-piridinil)-2(3H)-oxazolona; Metotrexato; Mitoxantrona, Heparina, Interferones; 2 macroglobulina-suero, ChIMP-3 (Pavloff, J. Bio. Chem. 267:17321-17326, (1992)); Quimostatina (Tomkinson, Biochem J. 286:475-480. (1992)); Tetradecasulfato de Ciclodextrina; Eponemicina; Camptotecina; Fumagillina (Ingber, Nature 348:555-557, (1990); Tiomalato de Sodio de Oro ("GST"; Matsabara y Ziff, J. Clin. Invest. 79:1440-1446, (1987)); anticolagenasa-suero; alfa2-antiplasmin (Holmes J. Biol. Chem. 262(4):1659-1664, (1987)); Bisantrene (National Cancer Institute); Lobenzarit disódico (ácido N-(2)-carboxifenil-4-cloroantronílico disódico o "CCA"; (Takeuchi, Agents Actions 36:312-316, (1992)), e inhibidores de la metaloproteinasa tales como BB94.

Factores anti-angiogénicos adicionales que también se pueden utilizar dentro del contexto del presente invento incluyen Talidomida (Celgene, Warren, NJ); esteroide antiestático; AGM-1470 (H. Brem y J. Folkman *J Pediatr. Surg.* 28:445-51 (1993)); un antagonista alfa integrina v beta 3 (C. Storgard, *J Clin. Invest.* 103:47-54 (1999)); carboxinaminolmidazol; carboxiamidotriazol (CAI) (Nacional Cáncer Institute, Bethesda, MD); Conbretastatina A-4 (CA4P) (OXIGENE, Boston, MA); Squalamina (Magaina Pharmaceuticals, Plymouth Meeting, PA); TNP-470, (Tap Pharmaceuticals, Deerfield, IL); ZD-0101 AstraZeneca (London, UK); APRA (CT2584); Benefin, Byrostatin-1 (SC339555); CGP-41251 (PKC 412); CM101; Dexrazoxane (ICRF187); DMXAA; Endostatin; Flavopridiol; Genesteina; GTE; ImmTher; Iressa (ZD1839); Octreotide (Somatostatina); Penretina; Penacilamina; Photopoint; PI-88; Prinomastat (AG-3340)G Purlylin; Suradista (FCE26644); Tamoxifen (Nolvadex); Tazaroteno; Tetratiomolibdato; Xeloda (Capecitabina); y 5-Fluorouracil.

Los agentes anti-angiogénicos que se pueden administrar en combinación con los compuestos del invento pueden funcionar a través de una variedad de mecanismos incluyendo, pero sin limitar a proteolisis inhibitoria de la matriz extracelular, bloqueo de la función de moléculas de adhesión de la matriz extracelular de células endoteliales, al antagonizar la función de inductores de angiogénesis tal como factores de crecimiento, e inhibir receptores de integrina expresados sobre células endoteliales proliferativas. Ejemplos de inhibidores anti-angiogénicos que interfieren con proteolisis de matriz celular y que se pueden administrar en combinación con las composiciones del invento incluyen, pero no están limitados a AG-3340 (Agouron, La Jolla, CA), BAY-12-9566 (Bayer, West Haven, CT), BMS-275291 (Bristol Myers Squibb, Princeton, NJ), CGS-27032A (Novartis, East Hanover, NJ), Marimastat (British Biotech, Oxford, UK), y Metastat (Aeterna, St-Foy, Quebec). Ejemplos de inhibidores anti-angiogénicos que actúan bloqueando la función de moléculas de adhesión de matriz extracelular de células endoteliales y los cuales se pueden administrar en combinación con las composiciones del invento incluyen, pero no están limitados a EMD-121974 (Merck KcgaA Darmstatdt, Alemania) y Vitaxin (Ixsys, La Jolla, CA/Medimmune, Gaithersburg, MD). Ejemplos de agentes anti-angiogénicos que actuan al antagonizar o inhibir directamente inductores de angiogénesis y los cuales se pueden administrar en combinación con las composiciones del invento incluyen, pero no están limitados a Angiozyme (Ribozyme, Boulder, CO), anticuerpo anti-VEGF (Genentech, S. San Francisco CA), PTK-787/ZK-225846 (Novartis, Basel, Suiza), SU-101 (Sugen, S. San Francisco, CA), SU-5416 (Sugen/Pharmacia Upjohn, Bridgewater, NJ), y SU-6668 (Sugen). Otros agentes anti-angiogénicos actúan para inhibir de manera indirecta angiogénesis. Ejemplos de inhibidores indirectos de angiogénesis que se pueden administrar en combinación con las composiciones del invento incluyen, pero no están limitados a IM-862 (Cytran, Kirkland, WA); Interferon-alfa, IL-12 (Roche, Nutley, NJ), y polisulfato de Pentosan (Georgetown University, Washington, DC).

En realizaciones particulares, se contempla el uso de composiciones del invento en combinación con agentes anti-angiogénicos para el tratamiento, prevención, y/o alivio de una enfermedad autoinmune, tal como por ejemplo una enfermedad autoinmune descrita en la presente.

En realizaciones particulares, se contempla el uso de composiciones del invento en combinación con agentes anti-angiogénicos para el tratamiento, prevención, y/o alivio de artritis. En una realización más particular, se contempla el uso de composiciones del invento en combinación con agentes anti-angiogénicos para el tratamiento, prevención, y/o alivio de artritis reumatoide.

En otra realización, los polipéptidos que codifican un polipéptido del presente invento son administrados en combinación con una proteína angiogénica, o polinucleotidos que codifican una proteína angiogénica. Ejemplos de proteínas angiogénicas que se pueden administrar con las composiciones del invento incluyen, pero no están limitados a factores de crecimiento de fibroblastos ácidos y básicos, VEGF-1, VEGF-2, VEGF-3, factor de crecimiento epidermal alfa y beta, factor de crecimiento de células endoteliales derivado de plaquetas, factor de crecimiento derivado de plaquetas, factor de necrosis tumoral alfa, factor de crecimiento de hepatocitos, factor de crecimiento tipo insulina, factor estimulador de colonias, factor estimulador de colonias de macrofagos, factor estimulador de colonias de granulocitos/macrofagos, y sintasa de óxido nítrico.

En realizaciones adicionales, las composiciones del invento se pueden administrar en combinación con un agente quimioterapéutico. Agentes quimioterapéuticos que se pueden administrar con las proteína de fusión de Albúmina y/o polinucleotidos del invento incluyen, pero no están limitados a agentes alquilantes tales como mostazas de nitrógeno (por ejemplo,

mecloretamina, ciclofosfamida ifosfamida de ciclofosfamida, melfalan (L-sarcolisina), y Clorambucil), etileniminas y metilmelaminas (por ejemplo, Hexametilmelamina y Tiotepa), alquilsulfonatos (por ejemplo, Busulfan), nitrosoureas (por ejemplo, carmustina (BCNU), Lomustina (CCNU), Semustina (metil-CCNU), Streptozocina (estreptozotocina)), triazenos (por ejemplo, Dacarbazina (DTIC; dimetiltriazenoimidazolcarboxamida)), análogos de ácido fólico (por ejemplo, metotrexato (ametopterina)), análogos de pirimidina (por ejemplo, Fluorouacil (5-fluorouracil; 5-FU); Floxuridina (fluorodesoxiuridina; FudR), y Citarabina (citosina arabinosida), análogos de purina e inhibidores relacionados (por ejemplo, Mercaptopurina (6-mercaptopurina; 6-MP), tioguanina (6-tioguanina; TG), y Pentostatina (2'-desoxicoformicina)), alcaloides vinca (por ejemplo, Vinblastina (VLB, sulfato de vinblastina)) y Vincristina (sulfato de vincristina)), epipodofilotoxinas (por ejemplo, Etoposida y Teniposida), antibióticos (por ejemplo, Dactinomicina (actinomicina D), Daunorubicina (daunomicina; rubidomicina), Doxorubicina, Bleomicina, Plicamicina (mitramicina), y Mitomicina (mitomicina C), enzimas (por ejemplo, L-asparaginasa), modificadores de respuesta biológica (por ejemplo, Interferón-alfa y interferón-alfa-2b), compuestos de coordinación de platino (por ejemplo, Cisplatin (cis-DDP) y Carboplatin), antracenediona (Mitoxantrona), ureas sustituidas (por ejemplo, Hidroxiurea), derivados de metilhidrazina (por ejemplo, Procarbazina (N-metilhidrazina; MIH), adrenocorticosteroides (por ejemplo, Prednisona), progestinas (por ejemplo, caproato de hidroxiprogesterona, medroxiprogesterona, acetato de medroxiprogesterona, y acetato de megestrol), estrógenos (por ejemplo, Dietilestilbestrol (DES), difosfato de dietilestrilbestrol (DES), difosfato de Dietilestrilbestrol, Estradiol, y estradiol de etinilo), antiestrógenos (por ejemplo, Tamoxifen), andrógenos (propionato de testosterona, y Fluoximesterona), antiandrógenos (por ejemplo, Tamoxifen), andrógenos (propionato de testosterona, y Fluoximesterona), antiandrógenos (por ejemplo, Flitamida), análogos de hormonas liberadoras de gonadotropina (por ejemplo, Leuprolida), otras hormonas y análogos de hormonas (por ejemplo, metiltestosterona, estramustina, fosfato sódico de estramustina, clorotrianiseno, y testolactona), y otras (por ejemplo, dicarbazina, ácido glutámico y mitotano).

En una realización, las composiciones del invento se administran en combinación con uno o más los siguientes fármacos: infliximab (también conocido como Remicade™ Centocor, Inc.), Trocade (Roche, RO-32-3555), Leflunomida (También conocido como Arava™ de Hoechst Marion Roussel), Kineret™ (un antagonista receptor de IL-1 también conocido como Anakinra de Amgen, Inc.).

En una realización específica, las composiciones del invento se administran en combinación con CHOP (ciclofosfamida, doxorubicina, vincristina, y prednisona) o combinaciones de uno o más de los compuestos de CHOP. En una realización, las composiciones del invento son administradas en combinación con anticuerpos anti-CD20, anticuerpos anti-CD20 monoclonales humanos. En otra realización, las composiciones del invento son administradas en combinación con anticuerpos anti-CD20 y CHOP, o anticuerpos anti-CD20 y cualquier combinación de uno o más componentes de CHOP, particularmente ciclofosfamida y/o prednisona. En una realización específica, las composiciones del invento son administradas en combinación con Rituximab. En una realización nacional, las composiciones del invento son administradas con Rituximab y CHPO, o Rituximab y cualquier combinación de uno o más de los componentes de CHOP, particularmente ciclofosfamida y/o prednisona. En una realización específica, las composiciones del invento son administradas en combinación con tositumomab. En una realización adicional, las composiciones del invento son administradas con tositumomab y CHOP, o tositumomab y cualquier combinación de uno o más de los componentes de CHOP,

particularmente ciclofosfamida y/o prednisona. Los anticuerpos anti-CD20 se pueden asociar de manera óptima con radioisótopos, toxinas o profármacos citotóxicos.

En otra realización específica, las composiciones del invento son administradas en combinación con Zevalin. En una realización adicional, las composiciones del invento son administradas con Zevalin y CHOP, o Zevalin y cualquier combinación de uno o más componentes de CHOP, particularmente ciclofosfamida y/o prednisona. Zevavin se puede asociar con uno o más radioisotopos. Isótopos particularmente preferidos son 90Y y 111In.

En una realización adicional, las proteínas de fusión de Albúmina y/o polinucleotidos del invento son administradas en combinación con citoquinas. Las citoquinas que se pueden administrar con las proteína de fusión de Albúmina y/o polinucleotidos del invento incluyen, pero no están limitadas a IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gama y TNF-alfa. En otra realización, las proteínas de fusión de albúmina y/o polinucleotidos del invento se pueden administrar con cualquier interleucina, incluyendo pero sin limitar a IL-1alfa, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, Il-18, Il-19, IL20, y IL-21.

En una realización, las proteína de fusión de albúmina y/o polinucleotidos del invento son administrados en combinación con miembros de la familia TNF. Moleculas TNF, TNF-relacionadas o tipo TNF que se pueden administrar con las proteínas de fusión de albúmina y/o polinucleotidos del invento incluyen, pero no están limitadas a formas solubles de TNF-alfa, linfotoxina-alfa (LT-alfa, también conocida como TNF-beta), LT-beta (encontrada en el heterotrimero LT-alfa2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gama (Publicación Internacional No. WO 96/14328), AIM-I (Publicación Internacional No. WO 97/33899), endoquina-alfa (Publicación Internacional No. WO 98/07880), OPG, y neutroquina-alfa (Publicación Internacional No. WO 98/18921, OX40, y factor de crecimiento nervioso (NGF), y formas solubles de Fas, CD30, CD27, CD40 y 4-IBB, TR2 (Publicación Internacional No. WO 96/34095), DR3 (Publicación Internacional No. WO 97/33904), DR4 (Publicación Internacional No. WO 98/32856), TR5 (Publicación Internacional No. WO 97/33904), DR4 (Publicación Internacional No. WO 98/32856), TR5 (Publicación Internacional No. WO 98/30693), TRANK, Tr9 (Publicación Internacional No. WO 98/56892), TR10 (Publicación Internacional No. WO 98/54202), 312C2 (Publicación Internacional No. WO 98/06842), y TR12, y formas solubles de CD154, CD70, y CD153.

En una realización adicional, las proteínas de fusión de Albúmina y/o polinucleotidos del invento son administradas en combinación con proteínas angiogénicas. Los proteínas angiogénicas que se pueden administrar con las proteínas de fusión de albúmina y/o polinucleotidos del invento incluyen, pero no están limitadas a Factor de Crecimiento Derivado de Glioma (GDGF), como se divulgo en el Numero de Patente Europea EP-399816; factor de crecimiento-A derivado de plaquetas (PDGF-A), como se divulga en la Patente Europea Número EP-682110; factor de crecimiento-B derivado de plaquetas (PDGF-B), como se divulga en la patente europea número EP-282317; factor de crecimiento placental (PIGF), como se divulga en la publicación internacional No. WO 92/06194; factor de crecimiento placental-2 (PIGF-2), como se divulga en Hauser, Factores de Crecimiento, 4:259-268 (1993); Factor de Crecimiento Endotelial Vascular (VEGF), como se divulga en la Publicación Internacional No. WO 90/13649; factor de crecimiento endotelial vascular-A (VEGF-A), como se divulga en la Patente Europea Número EP-506477; factor de crecimiento endotelial vascular-2 (VEGF-2) como se divulga en la publicación internacional No. WO 96/39515; factor de crecimiento endotelial vascular B (VEGF-3); factor de crecimiento endotelial vascular B-186 (VEGF-B186), como se divulga en la publicación internacional No. WO 96/26736; factor de crecimiento endotelial vascular-D (VEGF-

D), como se divulga en la publicación internacional No. WO 98/02543; factor de crecimiento endotelial vascular-D (VEGF-D), como se divulga en la publicación internacional No. WO 98/07832; factor de crecimiento endotelial vascular-E (VEGF-E), como se divulga en la patente alemana número DEI9639601. Las anteriores referencias mencionadas son incorporados en la presente como referencia en sus totalidades.

En una realización adicional, las proteína de fusión de Albúmina y/o polinucleotidos del invento son administrados en combinación con factores de crecimiento de fibroblastos. Los factores de crecimiento de fibroblastos que se pueden administrar con las proteínas de fusión de albúmina y/o polinucleotidos del invento incluyen, pero no están limitados a FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11m FGF-12, FGF-13, FGF-14 y FGF15.

En una realización adicional, las proteínas de fusión de Albúmina y/o polinucleotidos del invento son administrados en combinación con factores de crecimiento hematopoyeticos. Los factores de crecimiento hematopoyetico que se pueden administrar con las proteínas de fusión de Albúmina y/o polinucleotidos del invento incluyen, pero no están limitados a factor estimulador de colonias de granulocitos/macrofagos (GM-CSF) (sargramostim, LEUKINE, PROKINE), factor estimulador de colonias de granulocitos (G-CSF) (filgrastim, NEUPOGEN), factor estimulador de colonias de macrofagos (M-CSF, CSF-1) eritropoyetina (epoetin alfa, EPOGEN, PROCRIT), factor de células madre (SCF, ligando c-kit, factor de acero), factor estimulador de colonias de mecacariocitos, PIXY321 (una proteína de fusión GMCSF/IL-3), interleucinas, especialmente una o más de IL-1 a IL-12, interferón-gamma, o trombopoyetina.

En ciertas realizaciones, las proteínas de fusión de albúmina y/o polinucleotidos del presente invento son administrados en combinación con bloqueadores adrenérgicos, tales como, por ejemplo, acebutolol, atenolol, betaxolol, bisoprolol, carteolol, labetalol, metoprolol, nadolol, oxprenolol, penbutolol, pindolol, propanolol, sotalol, y timolol.

En otra realización, las proteínas de fusión de Albúmina y/o polinucleotidos del invento son administrados en combinación con un fármaco antiarrítmico (ej., adenosina, amidoarona, bretilio, digitalis, digoxina, digitoxina, diliazem, disopiramida, esmolol, flecainida, lidocaina, mexiletina, moricizina, fenitoina, procainamida, procainamida de N-acetilo, propafenona, propanolol, quinidina, sotalol, tocaidina, y verapamil).

En otra realización, las proteínas de fusión de Albúmina y/o polinucleotidos del invento son administrados en combinación con agentes diuréticos, tales como agentes inhibidores de anhidrasa carbónica (ej., acetazolamida, diclorfenamida, y metazolamida), diuréticos osmóticos (ej., glicerina, isosorida, y urea), diuréticos que exhiben Na+-K+-2Cl simporte (ej., furosemida, bumetanida, azosemida, piretanida, tripamida, ácido etacrínico, muzolimina, y torsemida), diuréticos de tiazida y tipo tiazida (ej., bendroflumetiazida, benztiazida, clorotiazida, hidroclorotiazida, hidroflumetiazida, meticlotiazida, politiazida, triclormetiazida, clortalidona, indapamida, metolazona, y quinetazona, diuréticos ahorradores de potasio (ej., amilorida y triamtereno), y antagonistas de receptores mineralcorticoides (ej., espironolactona, canrenona, y canrenoato de potasio).

En una realización, las proteínas de fusión de Albúmina y/o polinucleotidos del invento son administrados en combinación con tratamientos para trastornos de imbalance endocrino y/o hormonal. Los tratamientos para trastornos de imbalance endocrino y/o hormonal incluyen, pero no están limitados a ^{127}I, isótopos radioactivos de yodo tal como ^{131}I y ^{123}I; hormona de crecimiento recombinante, tal como HUMATROPE™ (samotropina recombinante); análogos de hormona de crecimiento tales como PROTROPIN™ (somatrem), agonistas de dopamina tales

como PARLODEL™ (bromocriptina); análogos de somatostatina tales como SANDOSTATINA™ (octreotida); preparaciones de gonadotropina tales como PREGNYL™, A.P.L. y PROFASI™ (godanotropina coriónica) (CG)), PERGONAL™ (menotropinas), y METRODIN™ (urololitropina (uFSH)); preparaciones de hormona de liberación de gonadotripona humana sintética tales como FACTREL y LUTREPULSE™ (hidrocloruro de gonadorelina); agonistas de gonadotropina sintética tales como LUPRON (acetato de leuprolida), SUPRELIN™ (acetato de histrelina), SYNAREL (acetato de nafarelina), y ZOLADEX™ (acetato de goserelina); preparaciones sintéticas de hormona liberadora de tirotropina tal como RELEFAC TRH y THYPINONA (protirelin); TSH humano recombinante tal como THYROGEN™; preparaciones sintéticas de sales de sodio de isómeros naturales de hórmonas tiroideas tales como L-T4, SYNTHROID™ y LEVOTHROID™ (sodio de levotiroxina), L-T3, CYTOMEL™ y TRIOSTAT™ (sodio de liotiroina), y THYROLAR™ (liotrix); compuestos antitiroideos tales como 6-n-propiltiouracilo (propiltiouracilo), 1-metil-2-mercaptoimidazol y TAPAZOLE™ (metimazol), NEO-MERCAZOLE™ (carbimazol); antagonistas receptores beta-adrenérgicos tales como propanolol y esmolol; bloqueadores de canales Ca2+; daxametasona y agentes de contraste radiológicos yodados tales como TELEPAQUE™ (ácido yopanóico) y ORAGRAFIN™ (ipodato de sodio).

Tratamientos adicionales para trastornos de imbalance endocrino y/o hormonal incluyen, pero no están limitados a estrógenos o estrógenos conjugados tales como ESTRACE (estradiol), ESTINYL (estradiol de etinilo), PREMARIN, ESTRATAB, ORTHO-EST, OGEN y estropitato (estrona), ESTROVIS (quinestrol), ESTRADERM (estradiol), DELESTROGEN y VALERGEN (valerato de estradiol), DEPO-ESTRADIOL CYPIONATE y ESTROJET LA (cipionato de estradiol); antiestrogenos tales como NOLVADEX (tamoxifen), SEROPHENE y CLOMID (clomifeno); progestinas tales como DURALUTIN (caproato de hidroxiprogesterona), MPA y DEPO-PROVERA (acetato de medroxiprogesterona), PROVERA y CYCRIN (MPA), MEGACE (acetato de megestrol), NORLUTIN (noretindrona), y NORLUTATE y AYGESTIN (acetato de noretindrono); implantes de progesterona tales como NORPLANT SYSTEM (implantes subdermales de norgestrel); antiprogestina tal como RU 486 (mifepristono); contraceptivos hormonales tales como ENOVID (noretinodrel mas mestranol), PROGESTASERT (dispositivo intrauterino que libera progesterona), LOESTRIN, BREVICON, MODICON, GENORA, NELONA, NORINYL, OVACON-35 Y OVACON-50 (estradiol/noretrindona de etinilo), LEVLEN, NORDETTE, TRI-LEVLEN y TRIPHASIL-21 (estradiol/levonorgestrel de etinilo) LO/OVRAL y OVRAL (estradiol/norgestrel de etinilo), DEMULEN (estradiol de etinilo/diacetato de etinodiol), NORINYL, ORTHO-NOVUM, NORETHIN, GENORA, y NELOVA (noretindrona/mestranol), DOSEGEN y ORTHO-CEPT (estradiol /dosogestrel de etinilo), ORTHO-CYCLEN y ORTHO-TRICYCLEN (estradiol(norgestimato de etinilo), MICRONOR Y NOR-QD (noretindrona), y OVRETE (norgestrel).

Tratamientos adicionales para trastornos de imbalance hormonal y/o endocrino incluyen, pero no están limitados a ésteres de testosterona, tales como acetato de metenolona y undecanoato de testosterona; androgenos parenterales y orales tales como TESTOJECT-50™ (testosterona), TESTEX™(propionato de testosterona), DELATESTRYL™(enantato de tosterona), DEPO-TESTOSTERONE™(cipionato de testosterona), DANOCRINE™(danazol), HALOTESTIN™ (fluoximesterona), ORETON METHYL™ y VIRILON™ (metiltestosterona), y OXANDRIN™ (oxandrolona); sistemas transdermales de testosterona tal como TESTODERM™; antagonista de receptor androgeno e inhibidores de 5-alfa reductasa tales como ANDROCUR™ (acetato de ciproterona), EULEXIN™ (flutamide), y PROSCAR™ (finasterida);

preparaciones hormonales tales como CORTROSYN™ (cosintropina); asteroides adrenocorticales y sus análogos sintéticos tales como ACLOVATE™ (dipropionato de alclometasona), CYCLOCORT™ (amcinonida), BECLOVENT™ y VANCERIL™ (dipropionato de beclometasona), CELESTONE™ (betametasona); y BENISONE™ y UTICORT™(benzoato de betametasona), DIPROSONE™(dipropionato de betametasona), CELESTONE PHOSPHATE™ (fosfato de sodio de betametasona) , CELESTONE SOLUSPAN™ (propionato de clobetasol), CLODERM™ (pivalato clocortolona), CORTEF™ y HIDROCORTONA™ (cortisol(hidrocortisona)), HYDROCORTONE ACETATE™ (cortisol(hidrocortisona)acetato), LOCOID™ (cortisol(hidrocortisona) butirato), HYDROCORTONE PHOSPHATE™ (cortisol(hidrocortisona)fosfato de sodio) , A-HYDROCODT™ y SOLUCORTEF™ (cortisol (hidrocortisona) succinato de sodio), WESTCORT™ (cortisol (hidrocortisona) valerato), CORTISONE ACETATE™ (acetato de cortisona), DESOWEN™ y TRIDESILON™ (desonida), TOPICORT™ (desoximetasona), DECADRON™ (dexametasona), DECADRON LA™ (acetato de dexametasona), DECADRON PHOSPHATE™ y HEXADROL PHOSPHATE™ (fosfato de sodio de dexametasona), FLORONE™ y MAXIFLOR™ (diacetato de diflorasona),. FLORRINEF ACETATE™ (acetato de fludrocortisona), AEROBID™ y NASALIDE™(flunisolida), FLUONID™ y SYNALAR™ (acetonida de fluocinolona), LIDEX™ (fluocinonida), FLUOR-OP™ y FML™ (fluorometolona), CORDRAN ™ (flurandrenolida), HALOG™ (halcinonida), HMS LIZUFILM™ (medrisona), MEDROL™ (metilprednisolona), DEPO-MEDROL™ y MEDROL ACETATE ™(acetato de metilprednisona), A-METHAPRED™ y SOLUMEDROL™ (succionato de sodio de metilprednisolona), ELOCON™ (furoato de mometasona), HALDRONE™ (acetato de parametasona), DELTA-CORTEF™ (prednisolona), ECONOPRED™ (acetato de prednisolona), HYDELTRASOL™ (fosfato de sodio de prednisolona), HYDELTRA-T.B.A™ (tebutato de prednisolona), DELTASONE™ (prednisona), ARISTOCORT™ y KENACORT™ (triamicinolona), KENALOG™ (acetonida y triamcinolona), ARISTOCORT™ y KENACORT DIACETATE™ (diacetato de triamsinolona), y ARISTOSPAN™ (hexacetonida de triamcinolona); inhibidores de biosíntesis y acción de esteroides adrenocorticales tales como CYTADREN (aminoglutehimida), NIZORAL ™(ketoconazola), MODRASTANE™ (trilostano), y METOPIRONE™ (metirapona); insulina bovina, porcina o humana o mezclas de las mismas; análogos de insulina; insulina humana recombinante tal como HUMULIN™ y NOVOLIN™; agentes hipoglicémicos orales tales como ORAMIDE™ y ORINASE™ (tolbutamida), DIABINESE™ (clorpropamida), TOLAMIDE y TOLINASE™ (tolazamida), DYMELOR™ (acetohexamida), glibenclamida, MICRONASE™, DIBETA™ y FLYNASE™ (gliburida), GLUCOTROL™ (glipizida), y DIAMICRON™ (gliclazida), GLUCOPHAGE™ (metformin), ciglitazona, pioglitazona, e inhibidores de la alfa glicosidasa; glucagon bovino o porcino; somatostatinas tales como SANDOSTATIN™ (OCTREOTIDA); y diazoxidas tales como PROGLYCEM™ (diazoxide).

En una realización, las proteínas de fusión de albúmina y/o polinucleotidos del invento son administrados en combinación con tratamientos para trastornos de motilidad uterina. Tratamientos para trastornos de motilidad uterina incluyen, pero no están limitados a fármacos de estrógenos tales como estrógenos conjugados (ej., PREMARIN® y ESTRATAB®), estradioles (ej., CLIMARA® y ALORA®), estropipato y clorotrianiseno; fármacos de progestina (ej., AMEN (medroxiprogesterona), MICRONOR® (acetato de noretidrona) PROMETRIUM® (progesterona y acetato de megestrol); en terapias de combinación de estrógenos/progesteronas

tales como, por ejemplo, estrógenos/ medroxiprogesterona conjugados (ej., PREMPRO® y PREMPHASE®) y acetato noretidrona/estradiol de etinilo (ej., FEMHRT).

En una realización adicional, las proteínas de fusión de albúmina y/o polinucleotidos del invento son administrados en combinación con fármacos efectivos para tratar de eficiencia de hierro y anemias hipocrómicas, incluyendo pero sin limitar a sulfato ferroso (sulfato de hierro, FEOSOL), fumarato ferroso (ej., FEOSTAT), gluconato ferroso (ej., FERGON), complejo de polisacarido y hierro (ej., NIFEREX), inyección de dextrana de hierro (ej., INFED), sulfato cúprico, piridoxina, Vitamina B12, inyección cianobalamina (ej., REDISOL, RUBRAMIN, PC), hidroxocobalamina, ácido fólico (ej., FOLVITE), leucovorina (ácido folínico, 5-CHOH4PteGlu, factor de citrovorum) o WELLCOVORIN (sal de calcio de leucovorin), transferrina o ferritina.

En ciertas realizaciones, las proteínas de fusión de albúmina y/o polinucleotidos del invento son administrados en combinación con agentes utilizados para tratar trastornos psiquiátricos. Fármacos psiquiátricos que se pueden administrar con las proteínas de fusión de albúmina y/o polinucleotidos del invento incluyen, pero no están limitados a agentes antipsicóticos (ej., clorpromazina, clorprotixeno, clozapina, flufenazina, haloperidol, loxapina, mesoridazina, molindona, olanzapina, perfenazina, pimozida, quetiapina, risperidona, tioridazina, tiotixeno, trifluoperazina, y triflupromazina), agentes antimánicos (ej., carbamazepina, sodio de divalproex, carbonato de litio y citrato de litio), antidepresivos (ej., amitriptilina, amoxapina, bupropion, citalopram, clomipramina, desipramina, doxepina, fluvoxamina, fluoxetina, imipramina, isocarboxazid, maprotilina, mirtazapina, nefazodona, nortriptilino, paroxetina, fenelzina, protriptilina, sertralina, tranilcipromina, trazodona, trimipramina, y venlafaxina), agentes antiansiedad (ej., alprazolam, buspirona, clordiazepoxido, clorazepato, diazepam, halazepam, lorazepam, y prazepam), y estimulantes (ej., d-anfetamina, metilfenidato, y pemolina).

En otras realizaciones, las proteínas de fusión de albúmina y/o polinucleotidos del invento son administrados en combinación con agentes utilizados para tratar trastornos neurológicos. Agentes neurológicos que se pueden administrar con las proteínas de fusión de albúmina y/o polinucleotidos del invento incluyen, pero no están limitados a agentes antiepilépticos (ej., carbamazepina, clonazepam, etosuximida, fenobarbital, fenitoina, primidona, ácido valproico, y sodio de divalproex, felbamato, gabapentina, lamotrigina, levetiracetam, oxcarbazepina, tiagabina, topiramato, zonisamida, diazepam, lorazepam, y clonazepam), agentes antiparquinsonianos (ej., levodopa/carbidopa, selegilina, amantidina, bromocriptina, pergolida, ropinirola, pramipexol, benztropina; biperiden, etopropazina, prociclinida, trihexifenidilo, tolcapona), terapéuticos ALS (ej., riluzola).

En otra realización, las proteínas de fusión de albúmina y/o polinucleotidos del invento son administrados en combinación con agentes vasodilatadores y/o agentes bloqueadores de canales de calcio. Agentes vasodilatadores que se pueden administrar con las proteínas de fusión de albúmina y/o polinucleotidos del invento incluyen, pero no están limitados a inhibidores de Enzimas de Conversión de Angiotensina (ACE) (ej., papaverina, isoxsuprina, benazepril, captopril, cilazapril, enalapril, enalaprilat, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, espirapril, trandolapril, y nilidrina), y nitratos (ej., dinitrato de isosoburo, mononitrato de isosorburo, y nitroglicerina). Ejemplos de agentes bloqueadores de canales de calcio que se pueden administrar en combinación con las proteínas de fusión de albúmina y/o polinucleotidos del invento incluyen, pero no están limitados a amlodipina, bepridil, ditiazem, felodipina, flunarizina, isradipina, nicardipina, nifedipina, nimodipina, y verapamil.

En ciertas realizaciones, las proteínas de fusión de albúmina y/o polinucleotidos del invento son administrados en combinación con tratamientos para trastornos gastrointestinales. Los tratamientos para trastornos gastrointestinales que se pueden administrar con las proteínas de fusión de albúmina y/o polinucleotidos del invento incluyen, pero no están limitados a antagonistas de receptores de histamina de H2 (ej., TAGAMET (cimetidina), ZANTAC (ranitinida), PEPCID (famotidina), y AXID (nizatidina)); inhibidores de H+, K+ ATPasa (ej., PREVACID (lansoprazol) y PRILOSEC (omeprazol)); compuestos de Bismuto (ej., PEPTO-BISMOL (subsalicitato de bismuto) y DE-NOL (bubcitrato de bismuto)); varios antacidos; sucralfato; análogos de prostaglandina (ej., CYTOTEC (misoprostol)); antagonistas colinérgicos muscarinicos; laxantes (ej., laxantes surfactantes, laxantes estimulantes, laxantes salinos y osmóticos); agentes antidiarréicos (ej., LOMOTIL (difenoxilato), MOTOFEN (difenoxin), y IMODIUM (hidrocloruro de loperamida), análogos sintéticos de somatostatina tales como SANDOSTATINA (octreotida), agentes antieméticos (ej., ZOFRAN (ondansetron), KYTRIL (hidrocloruro de granisetron), tropisetron, dolasetron, metoclopramida, clorpromazina, perfenazina, proclorperazina, prometazina, tietilperazina, trifluprornazina, domperidona, haloperidol, droperidol, trimetobenzamida, dexametasona, metilprednisolona, dronabinol, y nabilona); antagonistas D2 (ej., metoclopramida, trimetobenzamida, dexametasona, metilprednisolona, dronabinol. y nabilona); antagonistas de D2 (ej., metoclopramida, trimetobenzamida, y clorpromazina); sales biliares; ácido quenodesoxicólico; ácido ursodesoxicólico; y preparaciones de enzimas pancreáticas tales como pancreatina y pancrelipasa.

En realizaciones adicionales, las proteínas de fusión de albúmina y/o polinucleotidos del invento son administrados en combinación con otros regímenes terapéuticos o profilácticos, tal como, por ejemplo, terapia de radiación.

El invento también proporciona un kit o paquete farmacéutico que comprende uno o más recipientes llenados con uno o más ingredientes de las composiciones farmacéuticas que comprenden proteínas de fusión de albúmina del invento. Opcionalmente asociada con tales recipientes puede haber una nota en la forma prescrita por una agencia gubernamental que regula la fabricación, uso o venta de farmaceuticos o productos biológicos, tal nota refleja la aprobación de la agencia de la fabricación, utilización o venta par administración en humanos.

Terapia Genética

Constructos que codifican las proteínas de fusión de albúmina del invento se pueden utilizar como parte de un protocolo de terapia genética para liberar terapeuticamente dosis efectivas de las proteínas fusión de albúmina. Un enfoque preferido para introducción *in vivo* de ácido nucleico en una célula es el uso de un vector viral que contiene ácido nucleico, que codifica una proteína de fusión de albúmina del invento. La infección en células con un vector viral tiene la ventaja que una gran proporción de la células objetivo pueden recibir el ácido nucleico. Adicionalmente, las moléculas codificadas dentro del vector viral, ej., mediante un cADN contenido en el vector viral, son expresadas eficientemente en células las cuales han absorbido ácido nucleico del vector viral.

Los vectores de retrovirus y vectores de virus adeno-asociados se pueden utilizar con un sistema de liberación de genes recombinantes para la transferencia de moléculas de ácido nucleico exógeno que codifican proteínas de fusión de albúmina *in vivo*. Estos vectores proporcionan liberación eficiente de ácidos nucleicos en células, y los ácidos nucleicos transferidos son integrados de manera estable en el ADN cromosomal del huesped. El desarrollo de líneas celulares especializadas (denominadas "celulas de empaque") las cuales solamente producen retrovirus de replicación defectuoso ha aumentado la utilidad de retrovirus para terapia genética, y los retrovirus defectuosos se caracterizan por el uso en transferencia genética para

propósitos de terapia genética (para una reseña ver Miller, A.D. (1990) *Blood 76:27 1).* Un retrovirus de replicación defectuoso puede ser empacado en viriones los cuales se pueden utilizar para infectar una célula objetivo a través del uso de un virus auxiliar mediante técnicas estándares. Los protocolos para producir retrovirus recombinantes y para infectar células *in vitro* o *in vivo* con tales virus se pueden encontrar en Current Protocols in Molecular Biology, Ausubel. F. M. (eds) Greene Publishing Associates (1989), Secciones 9. 10-9.14 y otros manuales de laboratorio estándares.

Otro sistema de liberación de genes virales utiles en el presente invento utiliza vectores derivados de adenovirus. El genoma de un adenovirus se puede manipular de manera que codifique y exprese el producto genético de interés pero sea inactivado en términos de su capacidad para replicar un ciclo de vida viral lítico normal. Ver, por ejemplo, Berkner, *BioTechniques* 6:616 (1988); Rosenfeld, *Science* 252:431-434 (1991); Rosenfeld, *Cell* 68:143-155 (1992). Vectores adenovirales apropiados derivados de la cepa de adenovirus Ad type 5 d324 u otras cepas de adenovirus (ej., Ad2, Ad3, Ad7 etc) son conocidos por aquellos con experiencia en el estado de la técnica. Los adenovirus recombinantes pueden ser ventajosos en ciertas circunstancias puesto que no pueden infectar células no divisorias y se pueden utilizar para infectar una amplia variedad de tipos de células, incluyendo celulas epiteliales (Rosenfeld, (1992) citadas anteriormente). Además, la partícula viral es relativamente estable y tratable para purificación y concentración, y como antes, se puede modificar para afectar el espectro de infectividad. Adicionalmente, el ADN adenoviral introducido (y ADN extranjero contenido en el mismo) no es integrado en el genoma de una célula huesped pero permanece episomal, evitando así problemas potenciales que pueden ocurrir como resultado de mutagénesis de inserción en situaciones donde el ADN introducido se integra en el genoma huesped (*ej.*, ADN retroviral). Además, la capacidad de carga genética adenoviral para ADN extranjero es mayor (hasta ocho kilobases) con relación a otros vectores de liberación de genes (Berkner, citado anteriormente; Haj-Ahmand, J. Virol. 57:267 (1986)).

En otra realización, los sistemas de liberación de genes no virales del presente invento dependen de rutas entocíticas para la recaptación de la molécula nucleótida en cuestión mediante la célula escogida. Sistemas de liberación de genes de ejemplo de este tipo incluyen sistemas liposomáticas derivados, conjugados de poli-lisina, y envolventes virales artificiales. En una realización representativa, una molécula de ácido nucleico que codifica una proteína de fusión de albúmina del invento puede ser atrapada en liposomas que llevan cargas positivas sobre su superficie (ej., lipofectinas) y (opcionalmente) los cuales son etiquetados con anticuerpos contra antígenos de superficie celular del tejido objetivo (Mizuno (1992) *No Shinkei Geka* 20:547-5 5 1; Publicación PCT WO91/06309; solicitud de patente Japonesa 1047381; y solicitud de Patente Europea EP-A-43075).

Los sistemas de liberación de genes para un gen que codifica una proteína de fusión de albúmina del invento se pueden introducir en un paciente mediante cualquier número de métodos. Por ejemplo, una preparación farmacéutica para el sistema de liberación de genes se puede introducir de manera sistémica, ej., mediante inyección intravenosa, y la transducción específica de la proteína en las células objetivo ocurre predominantemente a partir de especificidad de transfección proporcionada por el vehículo de liberación de genes, expresión de tipo celular o tipo de tejidos debido a secuencias reguladoras transcripcionales que controlan expresión del gen receptor, o una combinación de los mismos. En otras realizaciones, la liberación inicial del gen recombinante es más limitada con la introducción en el animal siendo bastante localizada. Por ejemplo, el vehículo de liberación de genes se puede introducir mediante cateter (ver Patente Americana No. 5.328.470) o mediante inyección Estereostática (ej., Chen (1994) *PNAS 91: 3*

054-3 05 7). La preparación farmacéutica del constructo de terapia genética puede constar esencialmente del sistema de liberación de genes en un diluyente aceptable, o puede comprender una matriz de liberación lenta en la cual el vehículo de liberación de genes es incrustado. Donde la proteína de fusión de albúmina se puede producir intacta a partir de células recombinantes, ej. vectores retrovirales, la preparación farmacéutica puede comprender una o más células las cuales producen la proteína de fusión de albúmina.

Métodos Adicionales de Terapia Genética

también contemplados por invento están los métodos de terapia genética para tratar o prevenir trastornos, enfermedades y condiciones. Los métodos de terapia genética se relacionan con la introducción de secuencias de ácido nucleico (ADN, ARN y ADN o ARN antisentido) en un animal para lograr expresión de una proteína de fusión de albúmina del invento. Este método requiere un polinucleotido el cual codifica una proteína de fusión de albúmina del presente invento operativamente ligada a un promotor y cualquier otro elemento genético necesario para la expresión de la proteína fusión mediante el tejido objetivo. Tal terapia genética y técnicas de liberación son conocidas en el estado la técnica, ver, por ejemplo, WO90/11092, la cual es incorporada en la presente como referencia.

Así, por ejemplo, la células de un paciente se pueden diseñar con un polinucleotido (ADN o ARN) comprendiendo un promotor operablemente ligado a un polinucleotido que codifica una proteína de fusión de albumina del presente invento ex vivo, con las células modificadas luego siendo proporcionadas a un paciente a ser tratado con la proteína de fusión del presente invento. Tales métodos son bien conocidos en el estado de la técnica. Por ejemplo, ver Belldegrun, A. J. Natl. Cancer Inst. 85: 207-216 (1993); Ferrantini, M. Cancer Research 53: 1107-1112 (1993); Ferrantini, M. J. Immunology 153:4604-4615 (1994); Kaido, T. Int. J. Cancer 60: 221-229 (1995); Ogura, H. Cancer Research 50: 5102-5106 (1990); Santodonato, L., Human Gene Therapy 7:1-10 (1996); Santodonato, L. Gene Therapy 4:1246-1255 (1997); Zhang, J. F. Cancer Gene Therapy 3: 31-38 (1996), las cuales son incorporadas en la presente como referencia. En una realización, las células que son modificadas son células arteriales. Las células arteriales se pueden reintroducir en el paciente a través de inyección directa en la arteria, los tejidos que circundan la arteria, o a través de inyección de cateter.

Como se discute en detalle más adelante, los constructos polinucleotidos se pueden liberar mediante cualquier método que libera materiales inyectables en las células de un animal, tal como inyección en el espacio intersticial de tejidos (corazón, músculo, piel, pulmón, hígado, y similares). Los constructos polinucleotidos se pueden liberar en un líquido o vehículo acuoso farmacéuticamente aceptable.

En una realización, los polinucleotidos que codifican las proteínas de fusión de albúmina del invento son liberados como polinucleotidos desnudos. El término polinucleotido, ADN o ARN "desnudo" se refiere a secuencias que son libres de cualquier vehículo de liberacion que actua para asistir, promover o facilitar entrada en la célula, incluyendo secuencias virales, particulas virales, fórmulaciones de liposomas, lipofectina o agentes de precipitación y similares. Sin embargo, los polinucleotidos que codifican las proteínas de fusión de albumina del presente invento se pueden liberar en fórmulación de liposomas y fórmulaciones de lipofectina y se pueden preparar mediante métodos bien conocidos por aquellos con experiencia en el estado de la técnica. Tales métodos son descritos, por ejemplo, en las Patentes Americanas No. 5.593.972, 5.589.466 y 5.580.859, las cuales se incorporan en la presente como referencia.

Los constructos de vectores polinucleotidos utilizados en método de terapia genética probablemente son constructos que no se integrarán en el genoma huesped ni contendrán secuencias que permiten replicación. vectores apropiados incluyen pWLNEO, pSV2CAT,

pOG44, pXT1 y pSG disponibles en Stratagene; pSVK3, pBPV, pMSG y pSVL disponibles en Pharmacia; pEF1/V5, pcDNA3.1, y pRc/CMV2 disponibles en Invitrogen. Otros vectores apropiados serán facilmente aparentes para aquellos con experiencia en el estado de la técnica.

Cualquier promotor fuerte conocido por aquellos con experiencia en el estado la técnica se puede utilizar para impulsar la expresión de la secuencia polinucleotida. Promotores apropiados incluyen promotores adenovirales, tales como el promotor adenoviral mayor de expresión tardía; o promotores heterólogos, tales como el promotor de citomegalovirus (CMV); promotor de virus cincitial respiratorio (RSV); promotores inducibles, tal como el promotor MMT, el promotor de metalotioneina; promotores de choque térmico; el promotor de albúmina; el promotor ApoA1; protores de globina humana; promotores de quinasa de timidina viral, tal como el promotor de timidina quinasa del Herpes Simplex; LTRs retrovirales; el promotor de b-actina; y promotores de hormona de crecimiento humano. El promotor también puede ser el promotor nativo para el gen que corresponde a la porción de proteína Terapéutica de las proteínas de fusión de albúmina del invento.

A diferencia de otras técnicas de terapia genética, una ventaja principal de introducir secuencias de ácido nucleico en células objetivo es la naturaleza temporal de la síntesis polinucleotida en las células. Los estudios han mostrado que las secuencias de ADN no replicadoras se pueden introducir en células para proporcionar producción del polipéptido deseado durante periodos de hasta seis meses.

El constructo polinucleotido se puede liberar en el espacio intersticial de tejidos dentro de un animal, incluyendo tejidos de músculo, piel, cerebro, pulmón, hígado, bazo, médula ósea, timo, corazón, linfa, sangre, hueso, cartílago, páncreas, riñón, vesícula biliar, estómago, intestino, testículo, ovario, útero, recto, sistema nervioso, ojos, glándulas, y tejidos conectivos. El espacio intersticial de los tejidos comprende la matriz mucopolisacarida fluida intercelular entre las fibras reticulares de tejidos de órganos, fibras elásticas en las paredes de vasos o cámaras, fibras de colageno de tejidos fibrosos, o esa misma matriz dentro de tejido conectivo envainando células musculares o en las lagunas de hueso. Este es de manera similar el espacio ocupado por el plasma de la circulación y el fluido linfático de los canales limpatéticos. La liberación al espacio intersticial de tejido muscular es preferida por razones discutidas más adelante. Estos se pueden liberar de manera conveniente mediante inyección en los tejidos que comprenden estas células. Estos son preferiblemente liberados y expresados en células no divisorias persistentes las cuales son diferenciadas, aunque la liberación y expresión se pueda lograr en células no diferenciadas o menos completamente diferenciadas, tal como, por ejemplo, las células madre de sangre o fibroblastos de piel. Células musculares in vivo son particularmente competentes en capacidad para absorber y expresar polinucleotidos.

Para inyección de secuencias de ácidos nucleicos desnudos, una cantidad de dosificación efectiva de ADN o ARN oscilará entre 0.05 mg/kg de peso corporal y 50 mg/kg de peso corporal aproximadamente. Preferiblemente la dosificación estará entre 0.005 mg/kg y 20 mg/kg y más preferiblemente entre 0.05 mg/kg y 5 mg/kg aproximadamente. Naturalmente, como apreciará alguien con experiencia en el estado de la técnica, esta dosificación varía dependiendo del sitio de inyección de tejidos. La dosificación apropiada y efectiva de secuencia de ácido nucleico puede facilmente ser determinada por aquellos con experiencia en el estado de la técnica y puede depender de la condición tratada y la ruta de administración.

La ruta preferida de administración es la ruta parenteral de inyección en el espacio intersticial de tejidos. Sin embargo, también se pueden utilizar otras rutas parenterales, tales como, inhalación de una fórmulación de aerosol particularmente para liberación en los pulmones o tejidos bronquiales, garganta o membranas mucosas de la nariz. Además, los constructos de

ADN desnudo se pueden liberar en las anterias durante angioplastia mediante el cateter utilizado en el procedimiento.

Los polinucleotidos desnudos son liberados mediante cualquier método conocido en el estado de la técnica, incluyendo, pero sin limitar a inyección de aguja directa en el sitio de liberación, inyección intravenosa, administración tópica, infusión de cateter, y las conocidas "pistolas de genes". Estos métodos de liberación son conocidos en el estado la técnica.

Los constructos también se pueden liberar con vehículos de liberación tales como secuencias virales, partículas virales, fórmulaciones de liposomas, lipofectina, agentes de precipitación, etc. Tales métodos de liberación son bien conocidos en el estado de la técnica.

En ciertas realizaciones, los constructos polinucleotidos forman un complejo en una preparación liposomática. Las preparaciones liposomáticas para utilizar en el presente invento incluyen preparaciones catiónicas (cargadas positivamente), aniónicas (cargadas negativamente) y neutrales. Sin embargo, los liposomas catiónicos son particularmente preferidos puesto que se puede formar un complejo de carga ajustada entre el liposoma catiónico y el ácido nucleico polianiónico. Se ha mostrado que los liposomas catiónicos median en la liberación intracelular de ADN plásmido (Felgner, Proc. Natl. Acad. Sci USA (1987) 84:7413-7416, la cual se incorpora en la presente como referencia); mARN (Malone, Proc. Natl. Acad. Sci. USA (1989) 86:6077-6081, la cual se incorpora en la presente como referencia), en forma funcional.

Los liposomas catiónicos son facilmente disponibles. Por ejemplo, los liposomas de N[1-2,3-yoleiloxi)propil]-N,N,N-trietilamonio (DOTMA) son particularmente útiles y disponibles bajo la marca Lipofectin, de GIBCO BRL, Grand Island, N.Y. (ver, también, Felgner, Proc. Natl Acad. Sci. USA (1987) 84:7413-7416, la cual se incorpora en la presente como referencia). Otros liposomas comercialmente disponibles incluyen transfectasa (DDAB/POPE) y DOTAP/DOPE (Boechriger).

Otros liposomas catiónicos se pueden preparar a partir de materiales fácilmente disponibles utilizado técnicas bien conocidas en el estado de la técnica. Ver, ejemplo publicación PCT número WO 90/11092 (la cual es incorporada en la presente como referencia) para una descripción de la síntesis de liposomas DOTAP (1,2-bis(oleoiloxi)-3-(trimetilamonio)propano). La preparación de liposomas DOTMA es explicada en la literatura, ver, ej., P. Felgner, Proc. Natl. Acad. Sci USA 84:7413-7417, la cual es incorporada en la presente como referencia. Se pueden utilizar métodos similares para preparar liposomas a partir de otros materiales lípidos catiónicos.

De manera similar, los liposomas aniónicos y neutrales son fácilmente disponibles, tal como a partir de Avanti Polar Lipids (Birmingham, Ala.) o se pueden preparar fácilmente utilizando materiales fácilmente disponibles. Tales materiales incluyen fosfatidilo, colina, colesterol, etanolamina de fosfatidilo, colina de dioleoilfosfatidilo (DOPC), glicerol de dioleoilfosfatidilo (DOPG), etanolamina de dioleoilfafatidilo (DOPE), entre otros. Estos materiales también se pueden mezclar con los materiales de inicio DOTMA y DOTAP en proporciones apropiadas. Métodos para preparar liposomas utilizando estos materiales son bien conocidos en el estado de la técnica.

Por ejemplo, se pueden utilizar colina de dioleolfosfatidilo (DOPC), glicerol de dioleolfosfatidilo (DOPG), y etanolamina de dioleolfosfatidilo (DOPE) en varias combinaciones para preparar liposomas convencionales, con o sin la adición de colesterol. Así, por ejemplo, se pueden preparar vesículas de DOPG/DOPC al secar 50 mg de DOPG y DOPC bajo una corriente de gas de nitrógeno en un recipiente de sonicado. La muestra es colocada bajo una bomba de vacío durante la noche y es hidratada al día siguiente con agua desionizada. La muestra es luego sonicada durante 2 horas en un recipiente tapado, utilizando un sonicador Heat Systems modelo

350 equipado con una sonda de copa invertida (tipo baño) en la colocación máxima mientras el baño se hace circular a 15 grados centigrados. Alternativamente, las vesículas de carga negativa se pueden preparar sin sonicación para producir vesículas multilaminares o mediante extrusión a través de membranas con nucleoporos para producir vesículas unilaminares de tamaño discreto. Otros métodos son conocidos y disponibles para aquellos con experiencia en el estado de la técnica.

Los liposomas pueden comprender vesículas multilaminares (MLVs), vesículas unilaminares pequeñas (SUVs), o vesículas unilaminares grandes (LUVs), con SUVs como preferidas. Los diferentes complejos de liposomas-ácidos nucleicos son preparados utilizando métodos bien conocidos en el estado de la técnica. Ver, ej., Straubinger., Methods os Immunology (1983), 101:512-527, el cual es incorporado en la presente como referencia. Por ejemplo, MLVs que contienen ácidos nucleicos se pueden preparar al depositar una pelicula delgada de fosfolípido sobre las paredes de un tubo de vidrio y subsiguientemente hidratando con una solución del material a ser encapsulado. Las SUVs son preparadas mediante sonicación extendida de MLV para producir una población homóloga de liposomas unilaminares. El material a ser entrampado es agregado a una suspensión de MLVs preformadas y luego sonicado. Cuando se utilizan liposomas que contienen lípidos catiónicos, la pelicula lípida seca es resuspendida en una solución apropiada tal como agua estéril o una solución de amortiguador isotónico tal como 10 mM Tris/NaCl, sonicada, y luego los liposomas preformados son mezclados directamente con el ADN. El liposoma y ADN forman un complejo muy estable dedibo a unión de los liposomas de carga positiva con el ADN catiónico. SUVs encuentran uso con pequeños fragmentos de ácido nucleico. LUVs son preparadas mediante un número de métodos, bien conocidos en el estado de la técnica. Métodos comunmente utilizados incluyen quelación Ca2+-EDTA (Papahadjopoulos, Biochim, Biophys. Acta (1975) 394:483; Wilson, Cell 17:77 (1979)); inyección de éter (Deamer, D. y Bangham, A., Biochim. Biophys. Acta 443:629 (1976); Ostro, Biochem. Biophys. Res. Commun. 76:836 (1977); Fraley. Proc. Natl. Acad. Sci. USA 76:3348 (1979)); dialisis detergente (Enoch, H. Strittmatter. P., Proc. Nalt. Acad. Sci. USA 76:145 (1979)); y evaporación de fase inversa (REV) (Fraley, J. Biol. Chem. 255:10431 (1980); Szoka, F. y Papahadjooulos. D., Proc. Natl. Acad. Sci. USA 75:145 (1978); Schaefer-Ridder, Science 215:166 (1982)), las cuales son incorporadas en la presente como referencia.

Generalmente, la proporción de ADN a liposomas oscilará entre 10:1 y 1:10 aproximadamente. Preferiblemente, la proporción oscilará entre 5:1 y 1:5 aproximadamente. Más preferiblemente, la proporción oscilará entre 3:1 y 1:3 aproximadamente. Aún más preferiblemente, la proporción será 1:1 aproximadamente.

La patente americana No. 5.676.954 (la cual se incorpora en la presente como referencia) reporta la inyección del material genético, formando complejo con vehículos de liposomas catiónicos, en ratones Patentes Americanas No. 4.897.355, 4.946.787, 5.049.386, 5.459.127, 5.589.466, 5.693.622, 5.580.859, 5.703.055, y la publicación internacional No. WO 94/9469 (las cuales son incorporadas en la presente como referencia) proporcionan lípidos catiónicos para utilizar en transfección de ADN en células y mamíferos. Las Patentes Americanas No. 5.589.466, 5.693.622, 5.580.859, 5.703.055, y la publicación internacional No. WO 94/9469 proporcionan métodos para liberar complejos de ADN-lípidos catiónicos en mamíferos.

En ciertas realizaciones, la células son modificadas, *ex vivo* o *in vivo*, utilizando una particula retroviral que contiene ARN la cual comprende una secuencia que codifica una proteína de fusión de Albúmina del presente invento. Los retrovirus a partir de las cuales se pueden derivar los vectores plásmidos retrovirales incluyen, pero no están limitados a virus de la leucemia murina de moloney, virus de la necrosis de bazo, virus del sarcoma de Rous, virus del

sarcoma de Harvey, virus de la leucosis avícola, virus de la leucemia del mono Gibbon, virus de la inmunodeficiencia humana, virus del sarcoma mieloproliferativo, y virus del tumor mamario.

El vector plásmido retroviral se emplea para transducir líneas celulas de empaque para formar líneas de células productoras. Ejemplos de celulas de empaque que se pueden transfectar incluyen pero no están limitados a líneas celulares PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, y DAN tal como se describe en Miller, Human Gene Therapy 1:5-14 (1990), las cuales son incorporadas como referencia en su totalidad. El vector puede transducir las celulas de empaque a través de cualquier medio conocido en el estado de la técnica. Tales medios incluyen, pero no están limitados a electroporación, el uso de liposomas, y precipitación CaPO4. En una alternativa, el vector plásmido retroviral puede ser encapsulado en un liposomas, o acoplado a un lípido, y luego administrado a un huésped.

La línea de células productoras genera partículas de vectores retrovirales infecciosas las cuales incluyen polinucleotidos que codifican una proteína de fusión de Albúmina del presente invento. Luego tales partículas de vectores retrovirales se pueden emplear para transducir células eucarióticas, ya sea *in vitro* o *in vivo*. Las celulas eucarióticas transducidas expresarán una proteína de fusión del presente invento.

En ciertas realizaciones, la células son modificadas, *ex vivo* o *in vivo*, con polinucleotido contenido en un vector de adenovirus. El adenovirus se puede manipular de manera que codifique y exprese la proteína de fusión del presente invento, y al mismo tiempo es inactivado en términos de su capacidad para replicarse en un ciclo de vida viral lítico normal. La expresión de adenovirus es lograda sin integración del ADN viral en el cromosoma de la célula huésped, aliviando así preocupaciones acerca del mutagénesis de inserción. Además, los adenovirus han sido utilizados como vacunas entéricas vivas durante muchos años con un perfil de seguridad excelente (Schwartz. Am. Rev. Respir.Dis. 109:233.238 (1974)). Finalmente, la transferencia genética mediada por adenovirus ha sido demostrada en un número de instancias incluyendo transferencia de alfa-1-antitripsina y CFRT en los pulmones de ratas del algodón (Rosenfeld, M. A. (1991) Science 252:431-434; Rosenfeld (1992) Cell 68:143-155). Además, los estudios completos para intentar establecer adenovirus como un agente causante de cáncer humano fueron uniformemente negativos (Green, M. (1979) Proc. Natl. Acad. Sci USA 76:6606).

Vectores adenovirales adecuados útiles en el presente invento son descritos, por ejemplo, en Kozarsky y Wilson, Curre. Opin. Genet. Devel. 3:499-503 (1993); Rosenfeld, Cell 68:143-155 (1992); Engelhardt, Human Genet. Ther. 4:759-769 (1993); Yang, Nature Genet. 7:362-369 (1994); Wilson, Nature 365:691-692 (1993); y Patente Americana No. 5.652.224, las cuales son incorporadas en la presente como referencia. Por ejemplo, el vector de adenovirus Ad2 es útil y se puede desarrollar en células humanas 293. Estas células contienen la región E1 de adenovirus y constitutivamente expresan Ela y Elb, las cuales complementan los adenovirus defectuosos al proporcionar los productos de los genes eliminados desde el vector. Además de Ad2, otras variedades de adenovirus (ej., Ad3, Ad5, y Ad7) también son útiles en el presente invento.

Preferiblemente, los adenovirus utilizados en el presente invento son deficientes de replicación. Los adenovirus deficientes de replicación requieren la ayuda de un virus auxiliar y/o línea celular empaquetadora para formar partículas infecciosas. El virus resultante es capaz de infectar células y puede expresar un polinucleotido de interés el cual está operativamente ligado a un promotor, pero no puede replicarse en la mayoría de celulas. Los adenovirus deficientes de replicación pueden ser eliminados en una o más de todos o parte de los siguientes genes: E1a, E1b, E3, E2a, o L1 a L5.

En ciertas realizaciones, las celulas son modificadas ex vivo o *in vivo* utilizando un virus adeno asociado (AAV). Los AAVs son virus defectuosos de ocurrencia natural que requieren virus auxiliares para producir particulas infecciosas (Muzyeczca, N. Curr. Topic in Microbiol. Immunol. 158:97 (1992)). Este es uno de los pocos virus que pueden integrar su ADN en células no divisorias. Los vectores que contienen solamente 300 pares base de AAV se pueden empacar e integrar, pero el espacio para ADN exogeno es limitado a 4.5 kb aproximadamente. Los métodos para producir y utilizar tales AAVs son conocidos en el estado de la técnica. Ver, por ejemplo, las Patentes Americanas No. 5.139.941, 5.173.414, 5.354.414, 5.354.678, 5.436.146, 5.474.935, 5.478.745 y 5.589.377.

Por ejemplo, un vector AAV apropiado para utilizar en el presente invento incluirá todas las secuencias necesarias para replicación de ADN, encapsidación, e integración de celulas huésped. El constructo polinucleotido es insertado en el vector AAV utilizando métodos estándares de clonación, tales como aquellos encontrados en Sambrook, Clonación Molecular: Un Manual de Laboratorio, Cold Spring Harbor Press (1989). El vector AAV recombinante luego es transfectado en celulas empaquetadoras las cuales son infectadas con un virus auxiliar, utilizando cualquier técnica estándar, incluyendo lipofección, electroporación, precipitación de fosfato de cálcio, etc. Un virus auxiliar apropiado incluye adenovirus, citomegalovirus, virus vaccinia, o virus del herpes. Una vez las celulas empaquetadoras son transfectadas e infectadas, éstas producirán partículas virales AAV infecciosas las cuales contienen el constructo polinucleotido. Luego estas partículas virales son utilizadas para transducir celulas eucarióticas, ya sea ex vivo o in vivo. Las celulas transducidas contendrán el constructo polinucleotido integrado en su genoma, y expresarán una proteína de fusión del invento.

Otro método de terapia genética involucra regiones del control heterólogo y secuencias polinucleotidas endógenas operablemente asociadas (ej., que codifican un polipéptido del presente invento) a través de recombinación homologa (ver, patente americana No. 5.641.670, publicada en Junio 24 de 1997; publicación internacional No. WO 96/29411, publicada en Septiembre 26 de 1996; publicación internacional No. WO 94/12650, publicada en agosto 4 de 1994; Koller, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); y Zijlstra, Nature 342:435-438 (1989), las cuales son incorporadas en la presente como referencia. Este método involucra la activación de un gen el cual está presente en las celulas objetivo, pero el cual normalmente no es expresado en celulas, o es expresado a un nivel inferior que el deseado.

Los constructos polinucleotidos son hechos, utilizando técnicas estándares conocidas en el estado de la técnica, los cuales contienen el promotor con las secuencias objetivo flanqueando el promotor. Promotores apropiados son descritos en la presente. La secuencia objetivo es suficientemente complementaria a una secuencia endógena para permitir recombinación homóloga del promotor-secuencia objetivo con la secuencia endógena. La secuencia objetivo estará suficientemente cerca del extremo 5' de la secuencia polinucleotida endógena deseada de manera que el promotor estará operablemente ligado a la secuencia endógena ante recombinación homóloga.

El promotor y las secuencias objetivo se pueden amplificar utilizando PCR. Preferiblemente, el promotor amplificado contiene diferentes sitios de restricción enzimática sobre los extremos 5' y 3'. Preferiblemente, el extremo 3' de la primera secuencia objetivo contiene el mismo sitio de restricción enzimática que el extremo 5' del promotor amplificado y el extremo 5' de la segunda secuencia objetivo contiene el mismo sitio de restricción que el extremo 3' del promotor amplificado. El promotor amplificado y las secuencias objetivo son digeridas y ligadas entre sí.

El constructo de secuencia objetivo promotora es liberado en las celulas, ya sea como polinucleotido desnudo o en conjunción con agentes facilitadores de transfección, tales como liposomas, secuencias virales, partículas virales, virus completos, lipofección, agentes de precipitación, etc., descritos en detalle anteriormente. La secuencia objetivo promotora P se puede liberar mediante cualquier método, incluyendo inyección de aguja directa, inyección intravenosa, administración tópica, infusión de cateter, aceleradores de partículas, etc. Estos métodos son descritos en detalle más adelante.

El constructo de promotor-secuencia objetivo es absorbido por las células. La recombinación homóloga entre el constructo y la secuencia endógena toma lugar, tal que una secuencia endógena es colocada bajo el control del promotor. Luego el promotor impulsa la expresión de la secuencia endógena.

El polinucleotido que codifica una proteína de fusión de albúmina del presente invento puede contener una secuencia de señal de secreción que facilita la secreción de la proteína. Típicamente, la secuencia de señal es colocada en la región codificadora del polinucleotido a ser expresado hacia o en el extremo 5' de la región codificadora. La secuencia de señal puede ser homóloga o heteróloga al polinucleotido de interés y puede ser homóloga o heteróloga a las células a ser transfectadas. Adicionalmente, la secuencia de señal puede ser sintetizada de manera química utilizando métodos conocidos en el estado de la técnica.

Cualquier modo de administración de cualquiera de los constructos polinucleotidos antes descritos puede ser utilizado siempre y cuando el modo resulte en la expresión de una o más moléculas en la cantidad suficiente para proporcionar un efecto terapéutico. Esto incluye inyección de aguja directa, inyección sistémica, infusión de cateter, inyectores biolísticos, aceleradores de partículas (es decir, "pistolas de genes"), depósitos de esponja *gelfoam*, otros materiales de depósito comercialmente disponibles, bombas osmóticas, (ej., minibombas Alza) fórmulaciones farmacéuticas sólidas (tabletas o pastillas) orales o supositoriales, y aplicaciones decantadoras o tópicas durante cirugia. Por ejemplo, inyección directa de plásmido precipitado por fosfato de cálcio desnudo en hígado de rata y bazo de rata o un plásmido recubierto de proteína en la vena portal ha resultado en la expresión genética de gen extranjero en hígados de rata (Kaneda, Science 243:375 (1989)).

Un método preferido de administración local es mediante inyección directa. Preferiblemente, una proteína de fusión de albúmina del presente invento la cual se encuentra en complejo con un vehículo de liberación, es administrada mediante inyección directa en o localmente dentro del área de arterias. La administración de una composición localmente dentro del área de arterias se refiere a la inyección de la composición centímetros y preferiblemente, milímetros dentro de las arterias.

Otro método de administración local es contactar un constructo polinucleotido del presente invento en o alrededor de una herida quirúrgica. Por ejemplo, un paciente puede ser sometido a cirugía y el constructo polinucleotido puede ser esparcido sobre la superficie de tejido dentro de la herida o el constructo puede ser inyectado en áreas de tejido dentro de la herida.

Composiciones terapéuticas utiles en administración sistémica, incluyen proteínas de fusión del presente invento en complejo con un vehículo de liberación dirigida del presente invento. Vehículos de liberación apropiados para utilizar con administración sistémica comprenden liposomas que comprenden ligandos para dirigir el vehículo hacia un sitio particular. En realizaciones específicas, los vehículos de liberación adecuada para utilizar con administración sistémica comprenden liposomas que comprenden proteínas de fusión de albúmina del invento para dirigir el vehículo hacia un sitio particular.

Los métodos preferidos de administración sistémica, incluyen inyección intravenosa, liberación en aerosol, oral y percutánea (tópica). Las inyecciones intravenosos se pueden realizar utilizando métodos estándares en el estado de técnica. La liberación en aerosol también se pueden realizar utilizando métodos estándares en el estado de la técnica (ver, por ejemplo, Stribling, Proc. Natl. Acad. Sci. USA 189:11277-11281, 1992, la cual es incorporada en presente como referencia). La liberación oral se puede realizar al formar complejo de un constructo polinucleotido del presente invento con un vehículo capaz de resistir degradación mediante enzimas digestivas en los intestinos de un animal. Ejemplos de tales vehículos, incluyen cápsulas a tabletas plásticas, tales como aquellas conocidas en el estado de la técnica. La liberación tópica se puede realizar al mezclar un constructo polinucleotido del presente invento con un reactivo lipofílico (ej., DMSO) que es capaz de pasar hacia la piel.

La determinación de una cantidad efectiva de sustancia a ser liberada puede depender de un número de factores incluyendo, por ejemplo, la estructura química y actividad biológica de la sustancia, la edad y peso del animal, la condición exacta que requiere tratamiento y su severidad, y la ruta de administración. La frecuencia de tratamientos depende de un número de factores, tal como la cantidad de constructos polinucleotidos administrados por dosis, así como la salud e historial del sujeto. La cantidad precisa, número de dosis, y sincronización de dosis serán determinadas por el médico o veterinario a cargo.

Las proteínas de fusión de albúmina del presente invento se pueden administrar a cualquier animal, preferiblemente a mamíferos y aves. Mamíferos preferidos incluyen humanos, perros, gatos, ratones, ratas, conejos, ovejas, ganado, caballos y cerdos, con humanos como particularmente preferidos.

Actividades biológicas

Las proteínas de fusión de albúmina y/o polinucleotidos que codifican proteínas de fusión de albúmina del presente invento, se pueden utilizar ensayos para probar una o más actividades biológicas. Si una proteína de fusión de albúmina y/o polinucleotido exhibe una actividad en un ensayo particular, es probable que la proteína terapéutica que corresponde a una proteína de fusión pueda estar involucrada en enfermedades asociadas con la actividad biológica. Por lo tanto, la proteína de fusión se podría utilizar para tratar la enfermedad asociada.

En realizaciones preferidas, el presente invento contempla un método para tratar la enfermedad o trastorno listado en la columna "Indicación Preferida Y" de la Tabla 1 comprendiendo administración a un paciente en el cual tal tratamiento, prevención o alivio es deseado, una proteína de fusión de albúmina del invento que comprende la porción de proteína terapéutica que corresponde a una proteína terapéutica divulgada en la columna "proteína terapéutica X" de la Tabla 1 (en la misma fila que la enfermedad o trastorno a ser tratado es listado en la columna "indicación preferida Y" de la tabla 1) en una cantidad efectiva para tratar, prevenir o aliviar la enfermedad o trastorno.

En una realización preferida, el presente invento contempla un método para tratar la enfermedad o trastorno listado para una proteína terapéutica particular en la columna "indicación preferida: Y" de la Tabla 1 comprendiendo administración a un paciente en el cual tal tratamiento, prevención o alivio es deseado, una proteína de fusión de albúmina del invento que comprende una porción de proteína terapéutica que corresponde a la proteína terapéutica para la cual las indicaciones en los ejemplos están relacionadas en una cantidad efectiva para tratar, prevenir o aliviar la enfermedad o trastorno.

Específicamente contempladas por el presente invento están las proteínas de fusión de albúmina producidas mediante una célula cuando son codificadas por los polinucleotidos que codifican SEQ ID NO:Y. Cuando estos polinucleotidos se utilizan para expresar la proteína

codificada a partir de una célula, la secreción natural de una célula y pasos de procesamiento producen una proteína que carece de la secuencia de señal explícitamente listada en las columnas 4 y/o 11 de la tabla 2: la secuencia específica de aminoácidos de la secuencia de señal listada es mostrada en especificación o es bien conocida en el estado de la técnica. Así, las realizaciones más preferidas del presente invento incluyen la proteína de fusión de albúmina producida por una célula (la cual carecería de la secuencia guía mostrada en las columnas 4 y/o 11 de la tabla 2). También más preferidos son los polipéptidos que comprenden SEQ ID NO:Y sin la secuencia guía específica listada en las columnas 4 y/o 11 de la Tabla 2. Composiciones que comprenden estas dos realizaciones preferidas, incluyendo composiciones farmaceuticas, también son preferidas. Estas proteínas de fusión de albúmina son específicamente contempladas para tratar, prevenir, o aliviar una enfermedad o trastorno listado para una proteína terapéutica particular en la columna "Indicación Y Preferida" de la tabla 1.

En realizaciones preferidas, las proteínas de fusión del presente invento se pueden utilizar en la diagnosis, prognosis, prevención y/o tratamiento de enfermedades y/o trastornos relacionados con enfermedades y trastornos del sistema endocrino. (ver, por ejemplo, sección "trastornos endocrinos" mas adelante), el sistema nervioso (ver, por ejemplo, sección "trastornos neurológicos" más adelante), sistema respiratorio (ver, por ejemplo, sección "trastornos respiratorios" más adelante), sistema cardiovascular (ver, por ejemplo, sección "trastornos cardiovasculares" a continuación), enfermedades y/o trastornos relacionados con proliferación celular (ver, por ejemplo, sección "trastornos hiperproliferativos" más adelante), y/o trastornos con enfermedades relacionados con la sangre (ver, por ejemplo, la sección "trastornos relacionados con la sangre" más adelante).

En ciertas realizaciones, una proteína de fusión de albúmina del presente invento se puede utilizar para diagnosis y/o prognosis de enfermedades y/o trastornos asociados con los tejidos en los cuales el gen que corresponde a la porción de la proteína terapéutica de la proteína de fusión del invento es expresado.

Por lo tanto, las proteínas de fusión del invento y polinucleotidos que codifican proteínas de fusión de albúmina del invento son útiles en la diagnosis, detección y/o tratamiento de enfermedades y/o trastornos asociados con actividades que incluyen, pero no están limitadas a activación de prohormonas, actividad de neurotransmisores, señalización celular, proliferación celular, diferenciación celular, y migración celular.

Más generalmente, las proteínas de fusión del invento y polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para la diagnosis, prognosis, prevención y/o tratamiento de enfermedades y/o trastornos asociados con los siguientes sistemas.

Actividad Inmune

Las proteínas de fusión de albúmina del invento y polinucleotidos que codifican proteínas de fusión de albúmina del invento puede ser útiles para tratamiento, prevención, diagnosis y/o prognosis de enfermedades, trastornos, y/o condiciones del sistema inmune, mediante, por ejemplo, activación o inhibición de proliferación, diferenciación, o movilización de células inmunes (quimiotaxis). Las células inmunes se desarrollan a través de un proceso llamado hematopoyesis, produciendo células mieloides (plaquetas, glóbulos rojos, neurotrofilos, y macrofagos) y células linfoides (linfocitos B y T) a partir de células madre pluripotente. La etiología de estas enfermedades inmunes, trastornos, y/o condiciones puede ser genética, somática, tal como cancer y algunas enfermedades autoinmunes, adquirida (ej., mediante quimioterapia o toxinas), o infeccionsa. Además, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar como un marcador o detector de una enfermedad o trastorno particular del sistema inmune.

En otra realización, una proteína de fusión del invento y/o polinucleotido que codifica una proteína de fusión de albúmina del invento, se puede utilizar para tratar enfermedades o trastornos del sistema inmune y/o para inhibir o aumentar una respuesta inmune generada por células asociadas con los tejidos en los cuales el polipéptido del invento es expresado.

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para tratamiento, prevención, diagnosis, y/o prognosis de inmunodeficiencias, incluyendo inmunodeficiencias congénitas y adquiridas. Ejemplos de inmunodeficiencias humorales en donde los niveles de inmunoglobulinas, función humoral y/o cantidad de células B disminuyen, incluyen:: agamaglobulinemia X-ligada (enfermedad de Bruton), agamaglobulinemia infantil X-ligada, inmunodeficiencia X-ligada con hiper IgM, inmunodeficiencia no X-ligada con hiper IgM, síndrome linfoproliferativos X-ligado (XLP), agamaglobulinemia incluyendo agamaglobulinemia congénita y adquirida, agamaglobulinemia de inicio en el adulto, agamaglobulinemia de inicio tardio, disgamaglobulinemia, hipogamaglobulinemia, hipogamaglobulinemia no especificada, agamaglobulinemia recesiva (tipo Suizo), deficiencia IgM selectiva, deficiencia IgA selectiva, deficiencias de subclase IgG selectivas, deficiencia de subclase IgG (con o sin deficiencia IgA), deficiencia Ig con IgM aumentado, deficiencia IgG y IgA con IgM aumentado, deficiencia de anticuerpos con Igs normales o elevados, supresones de cadena pesada Ig, deficiencia de cadena kappa, trastorno linfoproliferativo de células B (BLPD), inmunodeficiencia variable común (CVID), inmunodeficiencia variable común (CVI) (adquirida), e hipogamaglobulinemia temporal de la infancia.

En realizaciones específicas, ataxia telangestasia o condiciones asociadas con ataxia telangestasia son tratadas, prevenidas, diagnosticadas y/o pronosticadas utilizando las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento.

Ejemplos de inmunodeficiencias congénitas en las cuales la función de células T y/o células B y/o número es disminuido incluyen, pero no están limitados a: anomalia de DiGeorge, inmunodeficiencias combinadas severas (SCID) (incluyendo, pero sin limitar a SCID X-ligado, SCID resesivo autosomal, deficiencia de adenosina deaminasa, deficiencia de nucleosida fosforilasa de purina (PNP), deficiencia de MHC Clase II (sindrome de linfocito Bare), síndrome Wiskott-Aldrich, y ataxia telangestasia), hipoplasia tímica, síndrome de tercera y cuarta bolsa faríngea, eliminación 22q11.2, candidiasis mococutánea crónica, deficiencia de células asesinas naturales (NK), linfocitopenia iodopática de células T CD4+; inmunodeficiencia con defecto de células T predominantes (sin especificar), e inmunodeficiencia no especificada de inmunidad mediada por células.

En realizaciones específicas, la anomalia de DiGeorge o condiciones asociadas con anomalia de DiGeorge son tratadas, prevenidas, diagnosticadas, y/o pronosticadas utilizando proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento.

Otras inmunodeficiencias que se pueden tratar, prevenir, diagnosticar, y/o pronosticar utilizando proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, incluyen, pero no están limitadas a enfermedad granulomatosa crónica, síndrome de Chédiak-Higashi, deficiencia de mieloperoxidasa, deficiencia leucocitaria de la glucosa-6-fosfato deshidrogenasa, síndrome linfoproliferativo X-ligado (XLP), deficiencia de adhesión de leucocitos, deficiencias de componente de complemento (incluyendo C1, C2, C3, C4, C5, C6, C7, C8 y/o deficiencias C9), disgenesis reticular, alinfoplasia-aplasia tímica, inmunodeficiencia con timoma, leucopenia congénita severa, displasia con inmunodeficiencia,

neutropenia neonatal, enanismo de extremidades cortas, inmunodeficiencia combinada de síndrome de Nezelof con Igs.

En una realización preferida, las inmunodeficiencias y/o condiciones asociadas con las inmunodeficiencias citadas anteriormente son tratadas, prevenidas, diagnosticadas y/o pronosticadas utilizando proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento.

En una realización preferida las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se podrían utilizar como un agente para aumentar la inmunosensibilidad entre individuos inmunodeficientes. En realizaciones específicas, las proteinas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar como un agente para aumentar la inmunosensibilidad entre individuos inmunodeficientes de células B y/o células T.

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para tratar, prevenir, diagnosticadar y/o pronosticar trastornos autoinmunes. Muchos trastornos autoinmunes resultan de un inadecuado autoreconocimiento como materia foránea por parte de las células inmunes. Éste reconocimiento inadecuado resulta en una respuesta inmune que lleva a la destrucción del tejido huésped. Por lo tanto, la administración de proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento que pueden inhibir una respuesta inmune, particularmente la proliferación, diferenciación, quimiotaxis de células-T, puede ser una terapia efectiva para prevenir trastornos autoinmunes.

Enfermedades o trastornos autoinmunes que se pueden tratar, prevenir, diagnosticar y/o pronosticar mediante proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen, pero no están limitados a uno o más de los siguientes: lupus sistémico eritematoso, artritis reumatoide, espondilitis anquilosante, esclerosis múltiple, tiroiditis autoinmune, tiroiditis de Hashimoto, anemia hemolítica autoinmune, anemia hemolítica, trombocitopenia, trombocitopenia autoinmune purpura, trombocitopenia autoinmune neonatal, trombocitopenia yodopática purpura, púrpura (ej., Henloch-Scoenlein púpura), autoinmunocitopenia, síndrome de Goodpasture, Penfigo vulgaris, miastenia gravis, enfermedad de Graves (hipertiroidismo), diabetes mellitus resistente a insulina.

Trastornos adicionales que probablemente tienen componentes autoinmunes que pueden ser tratados, prevenidos, y/o diagnosticados con las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen, pero no están limitados a artritis inducida por colageno tipo II, síndrome antifosfolípido, dermatitis, encefalomielitis alérgica, miocarditis, policondritis recurrente, enfermedad cardiaca reumática, neuritis, uveítis oftálmica, poliendocrinopatias, enfermedad de Reiter, Síndrome del hombre rígido, inflamación pulmonar autoinmune, autismo, síndrome de Guillain-Barre, diabetes mellitus dependiente de insulina, trastornos oculares inflamatorios autoinmunes.

Trastornos adicionales que probablemente tienen un componente autoinmune que pueden ser tratados, prevenidos, diagnosticados y/o pronosticados con las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen, pero no están limitados a escleroderma con anticuerpos anti-colageno (a menudo caracterizados, ej., por anticuerpos nucleolares y otros anticuerpos nucleares), enfermedad de tejido conectivo mixto (a menudo caracterizada, ej., por anticuerpos a antígenos nucleares extractables (ej., ribonucleoproteína)), polimiositis (a menudo caracterizada, ej., por nonhistona ANA), anemia perniciosa (a menudo caracterizada, ej., por célula antiparietal, microsomas, y anticuerpos de factor intrínseco), enfermedad yodopática de Addison (a menudo

caracterizada, ej., por citotoxicidad humoral y adrenal mediada por células, infertilidad (a menudo caracterizada, ej., por anticuerpos antiespermatozoales), glomerulonefritis (a menudo caracterizada, ej., por anticuerpos de membrana basal glomerular o complejos inmunes), penfigoide bulloso (a menudo caracterizado, ej., por IgG y complemento en membrana basal), síndrome de Sjogren (a menudo caracterizado, ej., por múltiples anticuerpos de tejidos, y/o ANA nonhistona específica (SS-B)), diabetes mellitus (a menudo caracterizada, ej., por anticuerpos de células de isletas humorales y mediados por células, resistencia a fármacos adrenérgicos (incluyendo resistencia a fármacos adrenérgicos con asma o fibrosis cística) (a menudo caracterizada, ej., por anticuerpos de receptores beta-adrenérgicos).

Trastornos adicionales que pueden tener un componente autoinmune que pueden ser tratados, prevenidos, diagnosticados y/o pronosticados con las proteínas de fusión de albúmina del presente invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen, pero no están limitados a, hepatitis activa crónica (a menudo caracterizada, ej., por anticuerpos de músculo liso), cirrosis biliar primaria (a menudo caracterizada, ej., por anticuerpos de mitocondrias), otra falla de glándula endocrina (a menudo caracterizada, ej., por anticuerpos de tejidos específicos en algunos casos), vitiligo (a menudo caracterizado, ej., por anticuerpos de melanocitos), vasculitis (a menudo caracterizada, ej., por Ig y complemento en paredes de vasos y/o complemento de suero bajo), most-MI (a menudo caracterizado, ej., por anticuerpos del miocardio), síndrome de cardiotomia (a menudo caracterizado, ej., por anticuerpos del miocardio), urticaria (a menudo caracterizada, ej., por anticuerpos IgG y IgM a IgE), dermatitis atopica (a menudo caracterizada por, ej., anticuerpos IgG y IgM a IgE), asma (a menudo caracterizada, ej., por anticuerpos IgG y IgM a IgE), y muchos otros trastornos inflamatorios, granulomatosos, degenerativos, y atróficos.

En una realización preferida, las enfermedades y trastornos autoinmunes y/o condiciones asociadas con las enfermedades y trastornos citados anteriormente son tratados, prevenidos, diagnosticados y/o pronosticados utilizando por ejemplo, proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento. En una realización preferida específica, la artritis reumatoide es tratada, prevenida, y/o diagnosticada utilizando proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión del invento.

En otra realización preferida específica, el lupus sistémico eritematoso es tratado, prevenido, y/o diagnosticado utilizando proteínas de fusión del invento y/o polinucleotidos que codifican las proteínas de fusión de albúmina del invento. En otra realización preferida específica, la trombocitopenia purpura yodopática es tratada, prevenida, y/o diagnosticada utilizando proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento.

En otra realización preferida específica, nefropatia IgA es tratada, prevenida, y/o diagnosticada utilizando proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento.

En una realización preferida, los trastornos y enfermedades autoinmunes y/o condiciones asociadas con las enfermedades y trastornos citados anteriormente son tratados, prevenidos, diagnosticados y/o pronosticados utilizando proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento.

En realizaciones preferidas, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento son utilizados como agentes inmunosupresivos.

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para tratar, prevenir, pronosticar,

y/o diagnosticar enfermedades, trastornos, y/o condiciones de células hematopoyeticas: las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser utilizados para aumentar diferenciación y proliferación de células hematopoyeticas, incluyendo células madre pluripotente, en un esfuerzo para tratar o prevenir aquellas enfermedades, trastornos, y/o condiciones asociadas con una disminución en ciertos tipos de células hematopoyeticas, incluyendo pero sin limitar a leucopenia, neutropenia, anemia, y trombocitopenia. Alternativamente, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para aumentar diferenciación y proliferación de células hematopoyeticas, incluyendo células madre pluripotente, en un esfuerzo para tratar o prevenir aquellas enfermedades, trastornos y/o condiciones asociados con un aumento en ciertos tipos de células hematopoyeticas, incluyendo pero sin limitar a histiocitosis.

Reacciones alérgicas y condiciones, tales como asma (particularmente asma alérgicas) u otros problemas respiratorios, también se pueden tratar, prevenir, diagnosticar y/o pronosticar utilizando proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento. Además, estas moléculas se pueden utilizar para tratar, prevenir, pronosticar, y/o diagnosticar anafilaxis, hipersensibilidad a una molécula antigénica, o incompatibilidad de grupos sanguíneos.

Adicionalmente, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se pueden utilizar para tratar, prevenir, diagnosticar y/o pronosticar reacciones alérgicas mediadas por IgE. Tales reacciones alérgicas incluyen, pero no están limitadas a asma, rinitis, y eczema. En realizaciones específicas, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser utilizados para modular concentraciones de IgE in vitro o in vivo.

Además, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento tiene cursos en la diagnosis, prognosis, prevención, y/o tratamiento de condiciones inflamatorios. Por ejemplo, puesto que las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden inhibir la activación, proliferación y/o diferenciación de células involucradas en una respuesta inflamatoria, estas moléculas se pueden utilizar para prevenir y/o tratar condiciones inflamatorias crónicas y agudas. Tales condiciones inflamatorias incluyen, pero no están limitadas a, por ejemplo, inflamación asociada con infección (ej., choque séptico, sepsis, o síndrome de respuesta inflamatoria sistémica), lesión de isquemia reperfusión, letalidad de endotoxinas, rechazo hiperagudo mediado por complemento, nefritis, lesión pulmonar inducida por citoquinas o quimioquinas, enfermedad de intestino inflamatorio, enfermedad de Crohn, sobreproducción de citoquinas (ej., TNF o IL-1), trastornos respiratorios (ej., asma y alergia); trastornos gastrointestinales (ej., enfermedad de intestino inflamatorio); cánceres (ej., gástrico, ovárico, pulmonar, de vejiga, hepático, y mamario); trastornos de CNS (sistema nervioso central) (ej., esclerosis múltiple; lesión cerebral isquémica y/o apoplejía, lesión cerebral traumática, trastornos neurodegenerativos (ej., enfermedad de Parkinson y enfermedad de Alzheimer; demencia relacionada con SIDA, y enfermedad por prion); trastornos cardiovasculares (ej., ateroesclerosis, miocarditis, enfermedad cardiovascular, complicaciones de desviación cardiopulmonar); así como también muchas enfermedades, condiciones, y trastornos adicionales que se caracterizan por inflamación (ej., hepatitis, artritis reumatoide, gota, trauma, pancreatitis, sarcoidosis, dermatitis, lesión de reperfusión de isquemia renal, enfermedad de Graves, lupus sistémico eritematoso, diabetes mellitus, rechazo de trasplantes alogénicos).

Ya que la inflamación es un mecanismo de defensa fundamental, los trastornos inflamatorios pueden afectar virtualmente cualquier tejido del cuerpo. Por consiguiente, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, tienen usos en el tratamiento de trastornos inflamatorios específicos a tejidos, incluyendo, pero sin limitar a adrenalitis, alveolitis, angiocolecistitis, apendicitis, balanitis, blefaritis, bronquitis, bursitis, carditis, celulitis, cervicitis, colecistitis, corditis, coclitis, colitis, conjuntivitis, cistitis, dermatitis, diverticulitis, encefalitis, endocarditis, esofagitis, eustachitis, fibrositis, foliculitis, gastritis, gingivitis, glositis, hepatoesplenitis, queratitis, labirintitis, laringitis, linfangitis, mastitis, otitis media, meningitis, metritis, miocarditis, miositis, miringitis, nefritis, neuritis, orquitis, osteocondritis, otitis, pericarditis, peritendonitis, peritonitis, faringitis, plebitis, poliomielitis, prostatitis, pulbitis, retinitis, rinitis, salpingitis, escleritis, esclerocoroiditis, escrotitis, sinusitis, espondilitis, esteatitis, estomatitis, sinovitis, siringitis, tendonitis, tonsilitis, uretritis, y vaginitis.

En realizaciones específicas, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, son útiles para diagnosticar, pronosticar, prevenir, y/o tratar rechazos de transplantes de órganos y enfermedad de injerto vs. huesped. El rechazo de órganos ocurre mediante destrucción de células inmunes de tejido trasplantado a través de una respuesta inmune. De manera similar, una respuesta inmune también está involucrada en GVHD, pero, en este caso, las células inmunes trasplantadas extrañas destruyen los tejidos huesped. Polipéptidos, anticuerpos, o polinucleotidos del invento, y/o agonistas o antagonistas de los mismos, que exhiben una respuesta inmune, particularmente la activación, proliferación, diferenciación, o quimiotaxis de células T, puede ser una terapia efectiva para prevenir rechazo de órganos, GVHD. En realizaciones específicas, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, que exhiben una respuesta inmune, particularmente la activación, proliferación, diferenciación, o quimiotaxis de células-T, puede ser una terapia efectiva para prevenir rechazo de xenoinjerto alérgico experimental e hiperagudo.

En otras realizaciones, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, son útiles para diagnosticar, pronosticar, prevenir, y/o tratar enfermedades de complejo inmune, incluyendo, pero sin limitar enfermedad de suero, glomerulonefritis post-estreptococal, poliarteritis nodosa, y vasculitis inducida por complejo inmune.

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para tratar, detectar, y/o prevenir agentes infecciosos. Por ejemplo, al aumentar la respuesta inmune, particularmente aumentando la proliferación, activación y/o diferenciación de células B y/o T, se pueden tratar, detectar, y/o prevenir enfermedades infecciosas. La respuesta inmune puede ser aumentada ya sea al aumentar una respuesta inmune existente, o al iniciar una nueva respuesta inmune. Alternativamente, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento también pueden inhibir directamente el agente infeccioso (se refiere a sección de aplicación que lista agentes infecciosos, etc), sin producir necesariamente una respuesta inmune.

En otra realización, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan como un coadyuvante de vacuna que aumenta la sensibilidad inmune a un antígeno. En una realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan como un coadyuvante para aumentar respuestas inmunes específicas a tumores.

En otra realizacion específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan como un coadyuvante para aumentar respuestas inmunes antivirales. La respuestas inmunes antivirales que se pueden aumentar utilizando composiciones del invento como un coadyuvante, incluyen virus y síntomas o enfermedades asociadas con virus descritos en la presente o de otra manera conocidas en el estado de la técnica. En realizaciones específicas, las composiciones del invento se utilizan como un coadyuvante para aumentar una respuesta inmune a un virus, enfermedad, o síntoma seleccionado del grupo que consta de: SIDA, meningitis, dengue, EBV, y hepatitis (ej., hepatitis B). En otra realización específica, las composiciones del invento se utilizan como un coadyuvante para aumentar la respuesta inmunes a un virus, enfermedad, o síntoma seleccionado del grupo que consta de VIH/SIDA, virus cincitial respiratorio, dengue, rotavirus, encefalitis japonesa B, influenza A y B, parainfluenza, paperas, citomegalovirus, hidrofobias, Junin, Chikungunia, Rift Valley Fever, herpes simplex, y fiebre amarilla.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan como un coadyuvante para aumentar respuestas inmunes anti-bacterianas o anti-micóticas. Las respuestas inmunes anti-bacterianas o anti-micóticas que se pueden aumentar utilizando composiciones del invento como un coadyuvante, incluyen enfermedades o síntomas asociados con hongos y bacterias descritos en la presente o de otra manera conocidos en el estado de la técnica. En realizaciones específicas, las composiciones del invento son utilizadas como un coadyuvante para aumentar una respuesta inmune a una bacteria u hongo, enfermedad, o síntoma seleccionado del grupo que consta de: tétanos, difteria, botulismo, y meningitis tipo B.

En otra realización específica, las composiciones del invento son utilizadas como un coadyuvante para aumentar la respuesta inmune a una bacteria u hongo, enfermedad, o síntoma seleccionado del grupo que consta de: *vibrio cholerae, Mycobacterium leprae, Salmonella typhi, Salmonella paratyphy, Meisseria meningitidis, Streptococcus pneumoniae,* estreptococus Grupo B, *Shigella spp., Escherichia coli* enterotoxigénica, *Escherichia coli* enterohemorrágica, y *Borrelia burgdorferi.*

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento son utilizadas como un coadyuvante para aumentar respuestas inmunes anti-parasitarias. Las respuestas inmunes anti-parasitarias que se pueden aumentar utilizando las composiciones del invento como un coadyuvante, incluyen parásitos y enfermedades o síntomas asociados con parásitos descritos en la presente o de otra forma conocidos en el estado de la técnica. En realizaciones específicas, las composiciones del invento se utilizan como un coadyuvante para aumentar una respuesta inmune a un parásito. En otra realización específica, las composiciones del invento se utilizan como un coadyuvante para aumentar la respuesta inmune a Plasmodium (malaria) o Leishmania.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento también se pueden emplear para tratar enfermedades infecciosas incluyendo silicosis, sarcoidosis, fibrosis pulmonar yodopática; por ejemplo, al prevenir el reclutamiento y la activación de fagocitos mononucleares.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan como un antígeno para la generación de un cuerpo para inhibir o aumentar respuestas mediadas inmunes contra polipéptidos del invento.

En una realización, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento son administrados a un animal (ej.,

ratón, rata, hamster, conejillo de Indias, cerdo, pollo, camello, cabra, caballo, vaca, oveja, perro, gato, primate no humano, y humano, preferentemente humano) para aumentar el sistema inmune para producir cantidades aumentadas de uno o más anticuerpos (ej.,g IgG, IgA, IgM, y IgE), para inducir producción de anticuerpos de mayor afinidad e intercambio de clases de inmunoglobulinas (ej., IgG, IgA, IgM, y IgE), y/o para aumentar una respuesta inmune.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan como un simulador de sensibilidad de células B a patógenos.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan como un activador de células T.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan como un agente que eleva la condición inmune de un individuo antes de recibir terapias inmunosupresivas.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina en invento se utilizan como un agente para inducir anticuerpos de mayor afinidad.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan como un agente para aumentar concentraciones de inmunoglobulina sérica.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan como un agente para acelerar recuperación de individuos inmunocomprometidos.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan como un agente para aumentar inmunosensibilidad entre poblaciones de edad avanzada y/o recién nacidos.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan como un aumentador del sistema inmune antes, durante, o después de transplantes de médula ósea y/o otros trasplantes (ej., transplantes de órganos alogenéicos o xenogenéicos). Con respecto a transplantes, las composiciones del invento se pueden administrar antes de, concomitantemente con, y/o después de transplantes. En una realización específica, las composiciones del invento son administradas después de transplantes, antes del comienzo de recuperación de poblaciones de células-T. En otra realización específica, las composiciones del invento son administradas primero después de transplante después del comienzo de recuperación de poblaciones de células T, pero antes de recuperación total de células B.

En otra realización específica, las proteínas de fusión de albumina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan como un agente para aumentar inmunosensibilidad entre individuos que tienen perdida adquirida de función de células B. Las condiciones que resultan en una pérdida adquirida de función de células B que se pueden mejorar o tratar mediante la administración de proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albumina del invento, incluyen, pero no están limitadas a infección de VIH, SIDA, transplante de médula ósea, lecucemia linfocitica crónica de células B (CLL).

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina de invento son utilizadas como un agente para aumentar inmunosensibilidad entre individuos que tienen una deficiencia inmune

temporal. Las condiciones que resultan en inmunodeficiencia temporal que pueden ser mejoradas o tratadas mediante la administración de proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, incluyen, pero no están limitadas a recuperación de infecciones virales (ej., influencia), condiciones asociadas con desnutrición, recuperación de mononucleosis infecciosa, o condiciones asociadas con estrés, recuperación de proteínas, recuperación de transfusión sanguínea, recuperación de cirugía.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan como un regulador de presentación de antígenos mediante monocitos, células dendríticas, y/o células B. En una realización, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento aumentan la presentación de antígenos o antagonizan presentación de antígenos in vivo o in vitro. Además, en realizaciones relacionadas, este aumento o antagonismo de presentación de antígenos puede ser útil como un tratamiento antitumoral para modular el sistema inmune.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan como un agente para dirigir el sistema inmune de un individuo hacia el desarrollo de una respuesta humoral (es decir TH2) opuesta a una respuesta celular TH1.

En otra realización específica, las proteínas de fusión de albúmina del presente invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del presente invento son utilizados como un medio para inducir proliferación tumoral y así haceria más susceptible a agentes anti-neoplásicos. Por ejemplo, mieloma múltiple es una enfermedad de división lenta y es por lo tanto refractaria a virtualmente todos los regimenes anti-neoplásicos. Si estas células fueron forzadas a proliferar con mayor rapidez su perfil de susceptibilidad probablemente cambiaría.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan como un estimulador de producción de células B en patologías tales como SIDA, trastorno de linfocitos crónico y/o Inmunodeficiencia Variable Común.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan como terapia para generación y/o regeneración de tejidos linfoides después de cirugía, trauma, o defecto genético. En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan en el pretratamiento de muestras de médula ósea antes de transplante.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican las proteínas de fusión de albúmina del invento se utilizan como una terapia basada en genes para trastornos genéticamente heredados que resultan en inmunocompetencia/inmunodeficiencia tal como se observa entre pacientes de SCID.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento son utilizados como un medio para activar monocitos/macrofagos para defender contra enfermedades parasitarias que afectan monocitos tales como Leismania.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina de invento son utilizados como un medio para regular citoquinas secretadas que son producidas por polipéptidos del invento.

En otra realización, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento son utilizados en una o más aplicaciones descritas en la presente, como aquellas que se pueden aplicar a medicina veterinaria.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento son utilizados como un medio para bloquear varios aspectos de respuestas inmunes a agentes extranjeros o así mismo. Ejemplos de enfermedades o condiciones en las cuales se desea el bloqueo de ciertos aspectos de respuestas inmunes pueden incluir trastornos autoinmunes tales como lupus, y artritis, así como también inmunosensibilidad alergias de piel, inflamación, enfermedad intestinal, lesión y enfermedades/trastornos asociados con patógenos.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan como una terapia para prevenir proliferación de células B y secreción Ig asociada con enfermedades autoinmunes tales como purpura trombocitopénica yodopática, lupus sistémico eritematoso y esclerosis múltiple.

En otra realización específica, los polipéptidos, anticuerpos, polinucleotidos y/o agonistas o antagonistas de las proteínas de fusión de invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento son utilizados como un inhibidor de migración de células B y/o T en células endoteliales. Esta actividad altera la arquitectura de tejidos o respuestas cognadas y es útil, por ejemplo para interrumpir respuestas inmunes, y la interrupción de la sepsis.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan como una terapia para hipogamaglobulinemia crónica evidente en enfermedades tales como gamopatia monoclonal de significado indeterminado (MGUS), enfermedad de Waldenstrom, gamopatias monoclonales yodopáticas relacionadas, y plasmacitomas.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden emplear por ejemplo para inhibir quimiotaxis polipéptida y activación de macrofagos y sus precursores, y de neurotrofilos, basofilos, linfocitos B y algunos subgrupos de células-T, ej., células T activadas y citotóxicas CD8 y células asesinas naturales, en ciertas enfermedades autoinmunes e inflamatorias crónicas e infecciosas. Ejemplos de enfermedades autoinmunes son descritos en la presente e incluyen esclerosis múltiple, y diabetes dependiente de insulina.

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento también se pueden emplear para tratar síndrome yodopático o híper-eosinofílico mediante, por ejemplo, prevención de migración y producción de eosinofilos.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan para aumentar o inhibir lisis celular mediada por complemento.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina de invento se utilizan para aumentar o inhibir citotoxicidad celular dependiente de anticuerpos.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento también se pueden emplear para tratar ateroesclerosis, por ejemplo, al prevenir filtración de monocitos en las paredes de arterias.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden emplear para tratar síndrome de distrés respiratorio del adulto (ARDS por su sigla en inglés).

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para estimular la curación de heridas y tejidos, estimular angiogénesis, y/o estimular curación de enfermedades o trastornos vasculares o linfáticos. Adicionalmente, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para estimular la regeneración de superficies mucosales.

En una realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan para diagnosticar, pronosticar, tratar, y/o prevenir un trastorno caracterizado por inmunodeficiencia primaria o adquirida, producción deficiente de inmunoglobulina sérica, infecciones recurrentes, y/o disfunción del sistema inmune. Además, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para tratar o prevenir infecciones de articulaciones, huesos, piel, y/o glándulas parótidas, infecciones transmitidas por sangre (ejemplo, sepsis, meningitis, artritis séptica, y/o osteomielitis), enfermedades autoinmunes (ejemplo, aquellas divulgadas en la presente), trastornos inflamatorios, y malignidades, y/o cualquier enfermedad o trastorno o condición asociada con estas infecciones, enfermedades, trastornos y/o malignidades) incluyendo, pero sin limitar a CVID, otras deficiencias inmunes primarias, enfermedad de VIH, CLL, bronquitis recurrente, sinusitis, otitis media, conjuntivitis, neumonía, hepatitis, meningitis, herpes zoster (ej., herpes zoster severo), y/o neumocistis carini. Otras enfermedades y trastornos que se pueden prevenir, diagnosticar, pronosticar, y/o tratar con proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen, pero no están limitados a infección por VIH, infección por HTLV-BLV, linfopenia, anemia de disfunción bactericidal de fagocitos, trombocitopenia, y hemoglobinuria.

En otra realización, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento son utilizados para tratar, y/o diagnosticar un individuo con enfermedad de inmunodeficiencia variable común ("CVID"; también conocida como "agamaglobulinemia adquirida" y "hipogamaglobulinemia adquirida") o un subgrupo de esta enfermedad.

En una realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para diagnosticar, pronosticar, prevenir, y/o tratar cánceres o neoplasmas incluyendo cánceres relacionados con células inmunes o tejidos inmunes o neoplasmas. Ejemplos de cánceres o neoplasmas que se pueden prevenir, diagnosticar, o tratar mediante proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen, pero no están limitados a leucemia mielógena, leucemia mielógena crónica, enfermedad de Hodgkin, linfoma no Hodgkin, anemia linfocítica aguda, leucemia linfocítica crónica, plasmacitomas, mieloma múltiple, linfoma de Burkitt, enfermedades EBV-transformadas, y/o enfermedades y trastornos descritos en la sección titulada "trastornos hiperproliferativos" en otra parte en la presente.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan como una terapia para disminuir proliferación celular de linfomas de células-B grandes.

En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento son utilizados como un medio para disminuir el envolvimiento de células B y Ig asociadas con Leucemia Mielógena Crónica.

En realizaciones específicas, las composiciones del invento se utilizan como un agente para aumentar inmunosensibilidad entre individuos inmunodeficientes de células B, tal que, por ejemplo, un individuo que ha experimentado una esplenectomía parcial o completa.

Trastornos Relacionados con Sangre

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para modular actividad hemostática (la detención de hemorragia) o trombolítica (disolución de coágulos). Por ejemplo, al aumentar la actividad hemostática o trombolítica, las proteínas de fusión de invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para tratar o prevenir enfermedades, trastornos, y/o condiciones de coagulación sanguínea (ej., afibrinogenemia, deficiencias de factor, hemofilia), enfermedades de plaquetas hemáticas, trastornos, y/o condiciones (ej., trombocitopenia), o heridas que resultan de trauma, cirugía, u otras causas. Alternativamente, las proteínas de fusión del invento de y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento que pueden disminuir actividad hemostática o trombolítica se podrían utilizar para inhibir o disolver coágulos. Estas moléculas podrían ser importantes del tratamiento o prevención de ataques cardiacos (infarto), apoplejías, o cicatrización.

En realizaciones específicas, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para prevenir, diagnosticar, pronosticar, y/o tratar trombosis, trombosis arterial, trombosis venosa, tromboembolismo, embolismo pulmonar, ateroesclerosis, infarto del miocardio, ataque isquémico temporal, angina inestable. En realizaciones específicas, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para prevención de oclusiones de injertos safenos, para reducir el riesgo de trombosis periprocedimiento como el que pudiera acompañar procedimientos de angioplastia, para reducir el riesgo de apoplejías en pacientes con fibrilación atrial incluyendo fibrilación atrial no reumática, para reducir el riesgo de embolismo asociado con enfermedad de válvulas cardiacas mecánicas de y/o de válvulas mitrales. Otros usos para proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, incluyen, pero no están limitados a la prevención de oclusiones en dispositivos extracorpóreos (ej., canulas intravasculares, derivaciones de acceso vascular en pacientes de hemodialisis, máquinas para hemodialisis y máquinas para derivación cardiopulmonar).

En otra realización, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se pueden utilizar para prevenir, diagnosticar, pronosticar, y/o tratar enfermedades y trastornos sanguíneos y/o órganos formadores de sangre asociados con los tejidos en los cuales el polipéptido del invento es expresado.

Las proteínas de fusión de invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para modular actividad hematopoyetica (la formación de células hemáticas). Por ejemplo, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para aumentar la cantidad de todas o subgrupos de células hemáticas, tal como, por ejemplo, eritrocitos, linfocitos (células B o T), células mieloides (ej., basofilos, eosinofilos, neutrofilos,

mastocitos, macrofagos) y plaquetas. La capacidad para disminuir la cantidad de células hemáticas o sus grupos de células hemáticas puede ser útil en la prevención, detención, diagnosis y/o tratamiento de anemias y leucopenias descritas más adelante. Alternativamente, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para disminuir la cantidad de todas o sus grupos de células hemáticas, tal como, por ejemplo, eritrocitos, linfocitos (células B o T), células mieloides (ej., basofilos, eosinofilos, neutrofilos, mastocitos, macrófagos) y plaquetas. La capacidad para disminuir la cantidad de células hemáticas o sus grupos de células hemáticas puede ser útil en la prevención, detención, diagnosis y/o tratamiento de leucocitosis, tal como, por ejemplo eosinofilia.

Las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para prevenir, tratar, o diagnosticar discrasia sanguínea.

Anemias son condiciones en las cuales el número de glóbulos rojos o cantidad de hemoglobina (la proteína que transporta el oxígeno) en éstos está por debajo de lo normal. La anemia se puede producir mediante sangrado excesivo, producción disminuida de glóbulos rojos, o destrucción aumentada de glóbulos rojos (hemolisis). Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para tratar, prevenir, y/o diagnosticar anemias. Las anemias que se pueden tratar, prevenir o diagnosticar mediante las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen anemia de deficiencia de hierro, anemia hipocrómica, anemia microcítica, clorosis, anemia sideroblástica hereditaria, anemia sideroblástica yodopática adquirida, aplasia del glóbulos rojos, anemia magaloblástica (ej., anemia perniciosa, (deficiencia de vitamina B12) y anemia de deficiencia de ácido fólico), anemia aplástica, anemias hemolíticas, (ej., anemia hololítica autoinmune, anemia hemolítica microangiopática, y hemoglobinuria nocturnal paroximal). Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para tratar, prevenir, y/o diagnosticar anemias asociadas con enfermedades incluyendo pero sin limitar a anemias asociadas con lupus sistémico eritematoso, cánceres, linfomas, enfermedad renal crónica, y bazos agrandados. Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para tratar, prevenir, y/o diagnosticar anemias provenientes de tratamientos farmacológicos tales como anemias asociadas con metildopa, dapsona, y/o sulfafármacos. Adicionalmente, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para tratar, prevenir, y/o diagnosticar anemias asociadas con arquitectura de glóbulos rojos anormal incluyendo pero sin limitar a esferocitosis hereditaria, eliptocitosis hereditaria, deficiencia de deshidrogenasa de glucosa-6-fosfato, y anemia de células falciformes.

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para tratar, prevenir, y/o diagnosticar anormalidades de hemoglobina, (ej., aquellas asociadas con anemia de células falciformes, enfermedad de hemoglobina C, enfermedad de hemoglobina S-C, y enfermedad de hemoglobina E). Adicionalmente, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para diagnosticar, pronosticar, prevenir, y/o tratar talasemias, incluyendo pero sin limitar la formas mayores y menores de alfa-talasemia y beta talasemia.

En otra realización, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para diagnosticar, pronosticar, prevenir, y/o tratar trastornos hemorrágicos incluyendo, pero sin limitar a trombocitopenia (ej., purpura trombocitopénica yodopática, y purpura trombocitopénica trombotica), enfermedad de Von Willebrand, trastornos de plaquetas hereditarias (ej., enfermedad por depósito tal como Chediak-Higashi y síndromes de Hermansky-Pudlak, disfunción de tromboxano A2, tromboastenia, y síndrome de Bernard-Soulier), síndrome hemolítico-urémico, hemofílias tal como hemofília A o deficiencia de Factor VII y hemofília B o deficiencia de factor IX, telangestasia hemorrágica hereditaria, también conocida como síndrome de Rendu-Osler-Weber, purpura alérgica (Henoch Schonlein purpura) y coagulación intravascular diseminada.

El efecto de las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento sobre el tiempo y coagulación de sangre puede ser monitoreado utilizando cualquiera de las pruebas de coagulación conocidas en el estado de la técnica incluyendo, pero sin limitar a tiempo de tromboplastina parcial sanguínea completa (PTT), el tiempo de tromboplastina parcial activada (aPTT), y el tiempo de coagulación activada (ACT), el tiempo de tromboplastina de coagulación activada recalcificada, o el tiempo de coagulación de Lee-White.

Varias enfermedades y una variedad de fármacos pueden producir disfunción de plaquetas. Por lo tanto, en la realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para diagnosticar, pronosticar, prevenir, y/o tratar disfunción de plaquetas adquirida tal como falla renal acompañada de disfunción de plaquetas, leucemia, mieloma múltiple, cirrosis hepática, y lupus sistémico eritematoso así como también disfunción de plaquetas asociadas con tratamientos farmacológicos, incluyendo tratamiento con aspirina, ticlopidina, fármacos anti-inflamatorios no esteroidales (utilizados para artritis, dolor, esguinces), y penicilina en altas dosis.

En otra realización, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para diagnosticar, pronosticar, prevenir, y/o tratar enfermedades y trastornos caracterizados por o asociados con números aumentados o disminuidos de glóbulos blancos. La leucopenia ocurre cuando el número de glóbulos blancos disminuye por debajo de lo normal. Las leucopenias incluyen, pero no están limitadas a neutropenia y linfocitopenia. Un aumento en el número de glóbulos blancos por encima de lo normal es conocido como leucocitosis. El cuerpo genera números aumentados de glóbulos blancos durante infección. Por lo tanto, la leucocitosis puede simplemente ser un parámetro fisiológico normal que refleja infección. Alternativamente, la leucocitosis puede ser un indicador de lesión u otra enfermedad tal como cáncer. Las leucocitosis incluyen pero no están limitadas a eosinofilia, y acumulaciones de macrofagos. En realizaciones específicas, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para diagnosticar, pronosticar, prevenir y/o tratar leucopenia. En otras realizaciones específicas, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para diagnosticar, pronosticar, prevenir, y/o tratar leucocitosis.

La leucopenia puede ser una disminución generalizada en todos tipos de glóbulos blancos, o puede ser una reducción específica de tipos particulares de glóbulos blancos. Por lo tanto, en realizaciones específicas, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para diagnosticar, pronosticar, prevenir, y/o tratar disminuciones en números de neurotrofilos, conocidas como

neutropenia. Las neutropenias que se pueden diagnosticar, pronosticar, prevenir, y/o tratar mediante las proteínas de fusión de albúmina de invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen, pero no están limitadas a agranulocitosis genética infantil, neutropenia familiar, neutropenia cíclica, neutropenias que resultan de o están asociadas con deficiencias de dieta (ej., deficiencia de vitamina B12 o deficiencia de ácido fólico), deficiencias que resultan de o están asociadas con tratamientos farmacológicos (ej., regímenes antibióticos tal como tratamiento de penicilina, tratamiento de sulfonamida, tratamiento anticoagulante, fármacos anticomvulsivos, fármacos antitiroideos, y quimioterapia para cáncer), y neutropenias que resultan de destrucción aumentada de neurotrofilos que puede ocurrir en asociación con algunas infecciones bacterianas o virales, trastornos alérgicos, enfermedades autoinmunes, condiciones en las cuales un individuo tiene un bazo agrandado (ej., síndrome de Felty, malaria y sarcoidosis), y algunos regímenes de tratamientos farmacológicos.

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para diagnosticar, pronosticar, prevenir, y/o tratar linfocitopenias (números disminuidos de linfocitos B y/o T), incluyendo, pero sin limitar a linfocitopenias que resultan de o están asociadas con estrés, tratamientos farmacológicos (tratamiento farmacológico con corticosteroides, quimioterapias de cáncer y/o terapias de radiación), infección de SIDA y/o otras enfermedades tales como, por ejemplo, cáncer, artritis reumatoide, lupus sistémico eritematoso, infecciones crónicas, algunas infecciones virales y/o trastornos hereditarios (ej., síndrome de DiGeorge, Síndrome de Wislott-Aldrich, inmunodeficiencia combinada severa, ataxia telangestasia).

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para diagnosticar, pronosticar, prevenir, y/o tratar enfermedades y trastornos asociados con números de macrofagos y/o función macrofaga incluyendo, pero sin limitar a enfermedad de Gaucher, enfermedad de Niemann-Pick, enfermedad de Letterer-Siwe y enfermedad de Hand-Schuller-Christian.

En otra realización, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para diagnosticar, pronosticar, prevenir, y/o tratar enfermedades y trastornos asociados con números de eosinofilos y/o función eosinofila incluyendo, pero sin limitar a síndrome hipereosinofílico yodopático, síndrome de eosinofilia-mialgia, enfermedad de Hand-Schuller-Christian.

En otra realización, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para diagnosticar, pronosticar, prevenir, y/o tratar leucemias y linfomas incluyendo, pero sin limitar a leucemia linfocítica aguda (linfoblástica) (ALL), leucemia mieloide aguda (linfocítica, mielógena, mieloblástica mielomonocítica), leucemia linfocítica crónica (ej., leucemias de células B, leucemias de células T, síndrome de Sezary, y leucemia de células vellosas), leucemia mielocitica crónica (mieloide, mielógena, o granulocítica, linfoma de Hodgkin, linfoma no Hodgkin, linfoma de Burkitt, y micosis fungoides.

En otras realizaciones, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para diagnosticar, pronosticar, prevenir, y/o tratar enfermedades y trastornos de células de plasma incluyendo, pero sin limitar a discrasias de células de plasma, gamaopatias monoclonales de significado indeterminado, mieloma múltiple, macroglobulinemia, macroglobulinemia de Waldenstrom, crioglobulinemia, y fenómeno de Raynaud.

En otras realizaciones, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para tratar, prevenir,

y/o diagnosticar trastornos linfoproliferativos, incluyendo pero sin limitar a policitemia vera, policitemia relativa, policitemia secundaria, mielofibrosis, mielofibrosis aguda, metaplasia mieloide agnogénica, trombocitemia (incluyendo trombocitemia primaria y secundaria) y leucemia mielocitica crónica.

En otras realizaciones, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles como un tratamiento antes de cirugía, para aumentar producción de células hemáticas.

En otras realizaciones, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles como un agente para aumentar la migración, fagocitosis, producción de superóxidos, citotoxicidad celular dependiente de anticuerpos de neurotrofilos, eosinofilos y macrofagos.

En otras realizaciones, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles como un agente para aumentar el número de células madre en circulación antes de féresis de células madre. En otra realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles como un agente para aumentar el número de células madre en circulación antes de féresis de plaquetas.

En otras realizaciones, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles como un agente para aumentar producción de citoquinas.

En otras realizaciones, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para prevenir, diagnosticar, y/o tratar trastornos hematopoyeticos primarios.

Trastornos hiperproliferativos

En ciertas realizaciones, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para tratar o detectar trastornos hiperproliferativos, incluyendo neoplasmas. Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden inhibir la proliferación de trastornos a través de interacciones directas o indirectas. Alternativamente, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden proliferar otras células las cuales pueden inhibir el trastorno hiperproliferativo.

Por ejemplo, al aumentar una respuesta inmune, se pueden tratar cantidades antigénicas particularmente crecientes del trastorno hiperproliferativo o al proliferar, diferenciar, o movilizar células T, se pueden tratar trastornos hiperproliferativos. Esta respuesta inmune puede ser aumentada ya sea al aumentar la respuesta inmune existente, o al inhibir una nueva respuesta inmune. Alternativamente, la disminución de una respuesta inmune también puede ser un método para tratar trastornos hiperproliferativos, tal como un agente quimioterapéutico.

Ejemplos de trastornos hiperproliferativos que se pueden tratar o detectar mediante proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen, pero no están limitados a neoplasmas localizados en: colon, abdomen, hueso, mama, sistema digestivo, hígado, páncreas, peritoneo, glándulas endocrinas (adrenales, paratiroideas, pituitarias, testículos, ovario, timo, tiroideas), ojos, cabeza y cuello, nervios (centrales y periféricos), sistema linfático, pelvis, piel, tejido suave, bazo, tórax, y tracto urogenital.

De manera similar, otros trastornos hiperproliferativos también se pueden tratar o detectar mediante proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de

albúmina del invento. Ejemplos de tales trastornos hiperproliferativos incluyen, pero no están limitados a leucemia linfoblástica aguda de la niñez, leucemia linfoblástica aguda, leucemia linfocítica aguda, leucemia mieloide aguda, carcinoma adrenocortical, cáncer hepatocelular del adulto (primario), cáncer hepático del adulto (primario), leucemia linfocítica aguda del adulto, leucemia mieloide aguda del adulto, enfermedad de Hodgkin del adulto, linfoma de Hodgkin del adulto, leucemia linfocítica del adulto, linfoma no Hodgkin del adulto, cáncer hepático primario del adulto, sarcoma de tejidos suaves del adulto, linfoma relacionado con SIDA, malignidades relacionadas con SIDA, cáncer anal, astrocitoma, cáncer del ducto biliar, cáncer de vejiga, cáncer óseo, glioma de tallo cerebral, tumores cerebrales, cáncer de mama, cáncer de uretra y pelvis renal, linfoma del sistema nervioso central (primario), linfoma del sistema nervioso central, astrocitoma cerebral, cáncer cervical, cáncer hepatocelular infantil (primario), cáncer hepático infantil (primario), leucemia linfoblástica aguda infantil, leucemia mieloide aguda infantil, glioma de tallo cerebral infantil, astrocitoma cerebelar infantil, astrocitoma cerebral infantil, tumores cerebrales germinales extracraneales infantiles, enfermedad de Hodgkin infantil, linfoma de Hodgkin infantil, glioma de ruta visual e hipotalámica infantil, leucemia linfoblástica infantil, meduloblastoma infantil, linfoma no Hodgkin infantil, tumores neuroectodermales primitivos pineales y anteriormentetentoriales infantiles, cáncer hepático primario infantil, rabdomiosarcoma infantil, sarcoma de tejidos suaves infantil, ruta visual infantil y glioma hipotalámico, leucemia linfocítica crónica, leucemia mielógena crónica, cáncer de colon, linfoma de células T cutáneo, carcinoma de células de isletas de páncreas endocrino, cáncer endometrial, ependimoma, cáncer epitelial, cáncer esofágico, sarcoma de Ewing y tumores relacionados, cáncer pancreatico exocrino, tumor extracraneal de células germinales, tumor extragonadal de células germinales, cáncer del ducto biliar extrahepático, cáncer ocular, cáncer de mama femenino, enfermedad de Gaucher, cáncer de vesícula biliar, cáncer gástrico, tumor carcinoide gastrointestinal, tumores gastrointestinales, tumores de células germinales, tumor trofoblástico gestacional, leucemia de células vellosas, cáncer de cabeza y cuello, cáncer hepatocelular, enfermedad de Hodgkin, linfoma de Hodgkin, hipergamaglobulinemia, cáncer hipofaríngeo, cánceres intestinales, melanoma intraocular, carcinoma de células de isletas, cáncer pancreatico de células de isletas, sarcoma de Kaposi, cáncer de riñón, cáncer laríngeo, cáncer de labios y cavidad oral, cáncer de hígado, cáncer de pulmón, trastornos linfoproliferativos, macroglobulinemia, cáncer de mama masculino, mesotelioma maligno, timoma maligno, meduloblastoma, melanoma, mesotelioma, cáncer de cuello escamoso primario oculto metastático, cáncer de cuello escamoso primario metastático, cáncer de cuello escamoso metastático, mieloma múltiple, mieloma múltiple/neoplasma de células de plasma, síndrome mielodisplásico, leucemia mielógena, leucemia mieloide, trastornos mieloproliferativos, cáncer de cavidad nasal y seno paranasal, cáncer nasofaringeo, neuroblastoma, linfoma no Hopkins durante el embarazo, cáncer de piel del tipo no melanoma, cáncer pulmonar de células pequeñas, cáncer de cuello escamoso metastático primario oculto, cáncer orofaríngeo, sarcoma fibroso osteo/maligno, osteosarcoma/histiocitoma fibroso maligno, osteosarcoma/histiocitoma fibroso maligno de hueso, cáncer epitelial ovárico, tumor de células germinales ováricas, tumor potencial maligno bajo ovárico, cáncer pancreatico, paraproteinemias, purpura, cáncer paratiroideo, cáncer de pene, feocromocitoma, tumor pituitario, neoplasma de células de plasma/mieloma múltiple, linfoma de sistema nervioso central primario, cáncer hepático primario, cáncer de próstata, cáncer rectal, cáncer de células renales, cáncer de uretra y pelvis renal, retinoblastoma, rabdomiosarcoma, cáncer de glándulas salivales, sarcomas de sarcoidosis, síndrome de Sézary, cáncer de piel, cáncer pulmonar de células pequeñas, cáncer del intestino delgado, sarcoma de tejidos blandos, cáncer de cuello escamoso, cáncer estomacal, tumores neuroectodermales

primitivos pineales y supratentoriales, linfoma de células T, cáncer testicular, timoma, cáncer tiroideo, cáncer de células transicionales de la uretra y pelvis renal, cáncer de uretra y pelvis renal transicional, tumores trofoblásticos, cáncer de células de uretra y pelvis renal, cáncer uretral, cáncer uterino, sarcoma uterino, cáncer vaginal, ruta visual y glioma hipotalámico, cáncer vulvar, macroglobulinemia de Waldenstrom, tumor de wilms, y cualquier otra enfermedad hiperproliferativa, además de neoplasia, localizada en un sistema de órganos listado anteriormente.

En otra realización alternativa, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento son utilizados para diagnosticar, pronosticar, prevenir, y/o tratar condiciones premalignas y para prevenir progresión a un estado neoplásico o maligno, incluyendo pero sin limitar a aquellos transtornos descritos anteriormente. Tales usos son indicados en condiciones conocidas o sospechadas de preceder a progresión a neoplasia o cáncer, en particular, donde el crecimiento celular neoplásico consta de hiperplásia, metaplasia, o más particularmente ha ocurrido displasia (para reseña de tales condiciones de crecimiento anormal, ver Robbins y Angell, 1976, Patología Básica, ed Ed., W. B. Saunders Co. Philadelphia, pp. 68-79.)

Hiperplasia es una forma de proliferación celular controlada, que involucra un aumento en número de células en un tejido u órgano, sin alteración significativa en estructura o función. Los trastornos hiperplásicos que se pueden diagnosticar, pronosticar, prevenir, y/o tratar con proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen, pero no están limitados a hiperplasia de nódulo linfático mediastinal, hiperplasia angiolinfoide con eosinofilia, hiperplasia melanocítica atípica, hiperplasia de células basales, hiperplasia de nódulo linfático gigante benigno, hiperplasia de cemento, hiperplasia adrenal congénita, hiperplasia sebacea congénita, hiperplasia cística, hiperplasia cística de la mama, hiperplasia de dentadura, hiperplasia ductal, hiperplasia endometrial, hiperplasia fibromuscular, hiperplasia epitelial focal, hiperplasia gingival, hiperplasia fibrosa inflamatoria, hiperplasia papilar inflamatoria, hiperplasia endotelial papilar intravascular, hiperplasia nodular de próstata, hiperplasia degenerativa nodular, hiperplasia pseudoepiteliomatosa, hiperplasia sebacea senil, e hiperplasia verrucosa.

Metaplasia es una forma de crecimiento celular controlado en el cual un tipo de célula adulta o completamente diferenciada sustituye otro tipo de célula adulta. Trastornos metaplásicos los cuales se pueden diagnosticar, pronosticar, prevenir, y/o tratar con proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión del invento incluyen, pero no están limitados a metaplasia mieloide agnogénica, metaplasia aprocrina, metaplasia atípica, metaplasia autoparenquimatosa, metaplasia de tejido conectivo, metaplasia epitelial, metaplasia intestinal, anemia metaplásica, osificación metaplásica, pólipos metaplásicos, metaplasia mieloide, metaplasia mieloide primaria, metaplasia mieloide secundaria, metaplasia escamosa, metaplasia escamosa de amnios, metaplasia mieloide sintomática.

La displasia frecuentemente es un predecesor del cáncer, y se encuentra principalmente en los epitelios, es la forma más desordenada de crecimiento celular no neoplásico, involucrando la pérdida de uniformidad celular individual y en la orientación arquitectónica de células. Las células displásicas a menudo tienen núcleos anormalmente grandes, profundamente teñidos, y exhiben pleomorfismo. La displasia caracteristicamente ocurre donde existe inflamación o irritación crónica. Trastornos displásicos que se pueden diagnosticar, pronosticar, prevenir, y/o tratar con proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen, pero no están limitados a displasia ectodermal anhidrótica, displasia anterofacial, displasia torácica asfixiante, displasia atriodigital, displasia

broncopulmonar, displasia cerebral, displasia cervical, displasia condroectodermal, displasia cleidocraneal, displasia ectodermal congénita, displasia craneodiaficial, displasia craneocarpotarsal, displasia craneometafisial, displasia de dentina, displasia diafisial, displasia ectodermal, displasia de esmalte, displasia encefalo-oftálmica, displasia epifisaria hemimelia, displasia epifisialis multiplex, displasia epifisialis puntata, displasia epitelial, displasia faciodigitogenital, displasia fibrosa familiar de mandíbulas, displasia familiar de pliegues blancos, displasia fibromuscular, displasia fibrosa de hueso, displasia ósea florida, displasia renal-retinal hereditaria, displasia ectodermal hidrótica, displasia ectodermal hipohidrótica, displasia tímica linfopénica, displasia mamaria, displasia mandibulofacial, displasia metaficial, displasia de mondini, displasia fibrosa monostótica, displasia mucoepitelial, displasia epifisial múltiple, displasia oculoauriculovertebral, displasia oculodentodigital, displasia oculovertebral, displasia ontogénica, displasia oftalmomandibulomélica, displasia cemental periapical, displasia fibrosa poliostótica, displasia espondiloepifisial pseudoacondroplástica, displasia espondiloepifisial, displasia retinal, displasia septo-óptica, displasia espondiloepifisial, displasia ventrículoradial.

Trastornos pre-neoplásicos adicionales los cuales se pueden diagnosticar, pronostican, prevenir, y/o tratar con proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen, pero no están limitados a trastornos disproliferativos benignos (ej., tumores benignos, condiciones fibrocísticas, hipertrofia de tejidos, pólipos intestinales, pólipos colon, y displasia esofágica), leucopenia, queratosis, enfermedad de Bowen, Piel de Granjero, queilitis solar, queratosis solar.

En otra realización, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se puede utilizar para diagnosticar y/o pronosticar trastornos asociados con los tejidos en los cuales el polipéptido del invento es expresado.

En otra realización, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento conjugadas a una toxina o un isotopo radioactivo, como se describe en la presente, se pueden utilizar para tratar cánceres y neoplasmas, incluyendo, pero sin limitar a aquellos descritos en la presente. En una realización preferida adicional, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento conjugadas a una toxina o isótopo radiactivo, como se describe en la presente, se pueden utilizar para tratar leucemia mielógena aguda.

Adicionalmente, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento puede afectar apoptosis, y por lo tanto, serían útiles para tratar un número de enfermedades asociadas con superviviencia celular aumentada o la inhibición de apoptosis. Por ejemplo, las enfermedades asociadas con supervivencia celular aumentada o la inhibición de apoptosis que podrían ser diagnosticadas, pronosticadas, prevenidas, y/o tratadas mediante polinucleotidos, polipéptidos, y/o agonistas o antagonistas del invento, incluyen cánceres (tales como linfomas foliculares, carcinomas con mutaciones p53, y tumores dependientes de hormonas, incluyendo, pero sin limitar a cáncer de colon, tumores cardiacos, cáncer pancreatico, melanoma, retinoblastoma, glioblastoma, cáncer pulmonar, cáncer intestinal, cáncer testicular, cáncer estomacal, neuroblastoma, mixoma, mioma, linfoma, endotelioma, osteoblastoma, osteoclastoma, osteosarcoma, condrosarcoma, adenoma, cáncer de mama, cáncer de próstata, sarcoma de Kaposi y cáncer ovárico); trastornos autoinmunes tales como, esclerosis múltiple, síndrome de Sjogren, tiroiditis de Hashimoto, cirrosis biliar, enfermedad de Behcet, enfermedad de Crohn, polimiositis, lupus sistémico eritematoso y glomerulonefritis inmune-relacionada y artritis reumatoide) e infecciones virales (tales como

virus del herpes, poxvirus y adenovirus), inflamación, enfermedad de injerto versus huesped, rechazo agudo de injertos, y rechazo crónico de injertos.

En realizaciones preferidas, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan para inhibir crecimiento, progresión, y/o metástasis de cánceres, en particular aquellos listados anteriormente.

Condiciones o enfermedades adicionales asociados con supervivencia celular aumentada que podrían ser diagnosticadas, pronosticadas, prevenidas, y/o tratadas mediante proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, incluyen, pero no están limitadas a progresión, y/o metástasis de malignidades y trastornos relacionados tales como leucemia (incluyendo leucemias agudas (ej., leucemia linfocítica aguda, leucemia mielocítica aguda (incluyendo mieloblástica, promielocítica, mielomonocítica, monocítica, y eritroleucemia)) y leucemias crónicas (ej., leucemia mielocítica crónica (granulocítica) y leucemia linfocítica crónica)), policitemia vera, linfomas (ej., enfermedad de Hodgkin y enfermedad no Hodgkin), mieloma múltiple, macroglobulinemia de Waldenstrom, enfermedad de cadena pesada, y tumores sólidos incluyendo, pero sin limitar a sarcomas y carcinomas tales como fibrosarcoma, mixosarcoma, liposarcoma, condrosarcoma, sarcoma osteogénico, cordoma, angiosarcoma, endoteliosarcoma, linfangiosarcoma, linfangioendoteliosarcoma, sinovioma, mesotelioma, tumor de Ewing, leimiosarcoma, rabdomiosarcoma, carcinoma de colon, cáncer pancreatico, cáncer de mama, cáncer ovárico, cáncer de próstata, carcinoma celular escamoso, carcinoma de células basales, adenocarcinoma, carcinoma de glándulas sudoríparas, carcinoma de glándulas sebaceas, carcinoma papilar, adenocarcinomas papilares, cistadenocarcinoma, carcinoma medular, carcinoma broncogénico, carcinoma de células renales, hematoma, carcinoma de ducto biliar, coriocarcinoma, seminoma, carcinoma embrional, tumor de Wilm, cáncer cervical, tumor testicular, carcinoma pulmonar, carcinoma pulmonar de células pequeñas, carcinoma de vejiga, carcinoma epitelial, glioma, astrocitoma, meduloblastoma, craneofaringioma, ependimoma, pinealoma, emangioblastoma, neuroma acústico, oligodendroglioma, menangioma, melanoma, neuroblastoma, y retinoblastoma.

Enfermedades asociadas con apoptosis aumentada que podrían ser diagnosticadas, pronosticadas, prevenidas, y/o tratadas mediante proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, incluyen SIDA; trastornos neurodegenerativos (tals como enfermedad de Alzheimer, enfermedad de Parkinson, esclerosis lateral amiotrófica, rinitis pigmentosa, degeneración cerebelar, y tumor cerebral o enfermedad asociada); trastornos autoinmunes (tales como, esclerosis múltiple, síndrome de Sjogren, tiroiditis de Hashimoto, cirrosis biliar, enfermedad de Behcet, enfermedad de Crohn, polimiositis, lupus sistémico eritematoso y glomerulonefritis inmune relacionada y artritis reumatoide) síndromes mielodisplásicos (tales como anemia aplástica), enfermedad de injerto versus huesped, lesión isquémica (tal como aquella causa mediante infarto del miocardio, apoplejía y lesión de reperfusión), lesión hepática (ej., lesión hepática relacionada con hepatitis, lesión de isquemia /reperfusión, colestosis (lesión de ducto biliar) y cáncer hepático); enfermedad hepática inducida por toxinas (tal como aquella causa por alcohol), choque séptico, cachexia y anorexia.

Trastornos y/o enfermedades hiperproliferativAs que se pueden diagnosticar, pronosticar, prevenir, y/o tratar mediante proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, incluyen, pero no están limitadas a neoplasmas localizados en el hígado, abdomen, hueso, mama, sistema digestivo, páncreas, peritoneo, glándulas endocrinas (adrenales, paratiroideas, pituitarias, testículos, ovario, timo, tiroides), ojos,

cabeza y cuello, sistema nervioso (central y periférico), sistema linfático, pelvis, piel, tejidos suaves, bazo, tórax, y tracto urogenital.

De manera similar, otros trastornos hiperproliferativos también se pueden diagnosticar, pronosticar, prevenir, y/o tratar mediante proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento. Ejemplos de tales trastornos hiperproliferativos incluyen, pero no están limitadas a hipergamaglobulinemia, trastornos linfoproliferativos, paraproteinemias, púrpura, sarcoidosis, síndrome de Sézary, macroglobulinemia de Waldenstrom, enfermedad de Gaucher, histiocitosis, y cualquier otra enfermedad hiperproliferativa además de neoplasia, localizada en un sistema de órganos listado anteriormente.

Otra realización preferida utiliza polinucleotidos que codifican proteínas de fusión de albúmina del invento para inhibir división celular aberrante, mediante terapia genética utilizando el presente invento, y/o fusiones de proteínas o fragmentos de las mismas.

Por lo tanto, el presente invento proporciona un método para tratar trastornos proliferativos de células al insertar en una célula de proliferación anormal un polinucleotido que codifica una proteínas de fusión de albúmina del presente invento, donde dicho polinucleotido representa dicha expresión.

Otra realización del presente invento proporciona un método para tratar trastornos proliferativos de células en individuos comprendiendo la administración de una o más copias genéticas activas del presente invento a una célula o células de proliferación anormal. En una realización preferida, los polinucleotidos del presente invento son un constructo de ADN comprendiendo un vector de expresión recombinante efectivo para expresar una secuencia de ADN que codifica dichos polinucleotidos. En otra realización preferida del presente invento, el constructo de ADN que codifica proteínas de fusión del presente invento es insertado en células a ser tratadas utilizando un retrovirus, o más preferentemente un vector adenoviral (ver G J. Nabel, PNAS 1999 96: 324-326, el cual es incorporado en la presente como referencia). En una realización más preferida, el vector viral es defectuoso y no transformará células no proliferativas, solamente células proliferativas. Además, en una realización preferida, los polinucleotidos del presente invento insertados en células proliferativas ya sea solos, o en combinación con o fusionados a otros polinucleotidos, luego se pueden modular a través de un estimulo externo (es decir, magnético, de molecula pequeña específica, químico, o administración farmacológica, etc), el cual actua sobre el promotor corriente arriba de dichos polinucleotidos para inducir expresión del producto proteico codificado. Como tal, el efecto terapéutico beneficioso del presente invento se puede modular expresamente (es decir, aumentar, disminuir, o inhibir expresión del presente invento) con base en dicho estimulo externo.

Los polinucleotidos del presente invento pueden ser útiles para reprimir expresión de antígenos o genes oncogénicos. Por "reprimir expresión de los genes oncogénicos" se entiende la eliminación de la transcripción del gen, la degradación del transcripto genético (ARN de premensaje), la inhibición de empalme, la destrucción del ARN mensajero, la prevención de las modificaciones post-traslacionales de la proteína, la destrucción de la proteína, o la inhibición de la función normal de la proteína.

Para administración local a células de proliferación anormal, los polinucleotidos del presente invento se pueden administrar mediante cualquier método conocido por aquellos con experiencia en el estado en técnica incluyendo, pero sin limitar a transfección, electroporación, microinyección de células, o en vehículos tales como liposomas, lipofectina, o como polinucleotidos desnudos, o cualquier otro método descrito en la especificación. El polinucleotido del presente invento se puede liberar mediante cualquier sistema de liberación de genes tal como,

pero sin limitar a vectores retrovirales (Gilboa, J. Virology 44:845 (1982); Hocke, Nature 320:275 (1986); Wilson., Proc. Natl. Acad. Sci. U.S.A. 85:3014), sistema de virus vaccinia (Chakrabarty, Mol. Cell Biol. 5:3403 (1985) o otros sistemas de liberación de ADN eficientes (Yates, Nature 313:812 (1985)) conocidos por aquellos con experiencia en el estado de la técnica. Estas referencias son de ejemplo solamente y se incorporan en la presente como referencia. Como el objeto de liberar o transfectar específicamente células las cuales son de proliferación anormal y células no divisorias suplementarias, es preferible utilizar un retrovirus, o sistema de liberación adenoviral (como se describió en el estado de la técnica y en otra parte en la presente) conocidos por aquellos con experiencia en el estado de la técnica. Puesto que la replicación de ADN huesped es requerida para integración de ADN retroviral, el retrovirus no podrá auto replicarse debido a la falta de los genes de retrovirus necesarios para su ciclo de vida. El uso de tal sistema de administración retroviral para polinucleótidos del presente invento enfocarán dicho gen y constructor hacia células proliferantes anormales y dejarán quietas a las células normales no divisorias.

Los polinucleotiodos del presente invento se pueden liberar a sitios de trastornos/enfermedades proliferativas de células en órganos internos, cavidades corporales y similares mediante el uso de dispositivos de imágenes utilizados para guiar una aguja de inyección directamente al sitio de la enfermedad. Los polinucleotidos del presente invento también se pueden administrar en sitios de enfermedad en el momento de la intervención quirúrgica.

Por "enfermedad proliferativa célular" se entiende cualquier enfermedad o trastorno humano o animal, que afecta cualquier órgano o combinación de órganos, cavidades, o partes del cuerpo, la cual se caracteriza por proliferaciones anormales locales simples o múltiples de células, grupos de células, o tejidos, ya sean benignos o malignos.

Cualquier cantidad de los polinucleotidos del presente invento se puede administrar siempre y cuando tenga un efecto biologicamente inhibitorio sobre la proliferación de las células tratadas. Además, es posible administrar más de un polinucleotido del presente invento de manera simultanea al mismo sitio. Por "biologicamente inhibitorio" se entiende inhibición de crecimiento parcial o total así como también disminuciones en la tasa de proliferación o crecimiento de las células. La dosis biológicamente inhibitoria se puede determinar al evaluar los efectos de los polinucleotidos del presente invento sobre el crecimiento celular proliferativo maligno o anormal objetivo en cultivo de tejidos, crecimiento tumoral en animales y cultivos de células, o cualquier otro método conocido por alguien con experiencia común en el estado de la técnica.

Además, las proteinas de fusión del invento y/o polinucleotidos que codifican proteinas de fusión de albumina del invento son útiles para inhibir angiogenesis de tejidos o células proliferativas, ya sea solas, como una fusión de proteínas, o en combinación con otros polipéptidos directa o indirectamente, como se describe en la presente. En una realización más preferida, dicho efecto anti-angiogenesis se puede lograr indirectamente, por ejemplo, a través de inhibición de células hematopoyecticas, células específicas a tumores, tales como macrofagos específicos a tumores (Ver Joseph 1B, J Natl Cancer Inst, 90(21):1648-53 (1998), el cual es incorporado en la presente como referencia).

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para inhibir células o tejidos proliferativos a través de inducción de apoptosis. Estas proteínas de fusión y/o polinucleotidos pueden actuar ya sea directamente o indirectamente para inducir apoptosis de células y tejidos proliferativos, por ejemplo en la activación de un receptor de dominio de muerte, tal como receptor-1 de factor de necrosis tumoral (TNF), CD95 (Fas(APO-1), proteína mediada por

apoptosis relacionada con receptor TNF (TRAMP) y receptor-1 y -2 de ligando de inducción de apoptosis TNF relacionado (TRAIL) (ver Schulze-Oshoff K, Eur J. Biochem 254(3):439-59 (1998), la cual es incorporada en la presente como referencia). Además, en otra realización preferida del presente invento, estas proteínas de fusión y/o polinucleotidos pueden inducir apoptosis a través de otros mecanismos, tal como en la activación de otras proteínas las cuales activarán apoptosis, o a través de estimulación de la expresión de estas proteínas, ya sea solas o en combinación con coadyuvantes o fármacos de moleculas pequeñas, opoptonina,g galectinas, tioredoxinas, proteínas antiinflamatorias (ver por ejemplo, Mutat Res 400(1-2):447-55 (1998), Med Hypotheses. 50(5):423-33 (1998), Chem Biol Interact. Apr 24; 111-112:23-34 (1998); J mol Med. 76(6):402-12 (1998), Int J Tissue React; 20(1):3-15 81998), las cuales son incorporadas en la presente como referencia).

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento son útiles para inhibir la metástasis de células o tejidos proliferativos. La inhibición puede ocurrir como resultado directo de administración de estas proteínas de fusión de albúmina y/o polinucleotidos, o indirectamente, tal como activación de la expresión de proteínas conocidas para inhibir metástasis, por ejemplo integrinas alfa 4, (Ver, ej., Curr Top Microbiol Immnunol 1998; 231:125-41, la cual es incorporada en la presente como referencia). Tales efectos terapeuticos del presente invento se pueden lograr ya sea solos, o en combinación con coadyuvantes o fármacos de moleculas pequeñas.

En otra realización, en invento proporciona un método para liberar composiciones que contienen las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento a células objetivo que expresan un polipéptido unido por, que se une a, o se asocia con una proteína de fusión de albúmina del invento. Las proteínas de fusión de albúmina del invento pueden ser asociadas con polipéptidos heterólogos, ácidos nucleicos heterólogos, toxinas, o profármacos a través de interacciones hidrofóbicas, hidrofílicas, iónicas y/o covalentes.

Las proteínas de fusión de albúmina del invento son útiles para aumentar la inmunogenicidad y/o antigenicidad de tejidos o células proliferativos, ya sea directamente, tal como ocurriría si las proteínas de fusión de albúmina del invento "vacunaran" la respuesta inmune para responder a antígenos e inmunogenos proliferativos, o indirectamente, tal como en activación de la expresión de proteínas conocida por aumentar la respuesta inmune (ej., quimioquinas), a dichos antígenos e inmunogenos.

Trastornos renales

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se puede utilizar para tratar, prevenir, diagnosticar y/o pronosticar trastornos del sistema renal. Trastornos renales que se puede diagnosticar, pronosticar, prevenir, y/o tratar con composiciones del invento incluyen, pero no están limitados a, falla renal, nefritis, trastornos de vasos sanguíneos del riñón, trastornos renales metabólicos y congénitos, trastornos urinarios del riñón, trastornos autoinmunes, esclerosis y necrosis, imbalance electrolítico, y cánceres renales.

Las enfermedades renales que se puede diagnosticar, pronosticar, prevenir, y/o tratar con composiciones del invento incluyen, pero no están limitadas a falla renal aguda, falla renal crónica, falla renal ateroembólica, enfermedad renal terminal, enfermedades inflamatorias del riñon (ej., glomerulonefritis aguda, glomerulonefritis postinfecciosa, glomerulonefritis rápidamente progresiva, síndrome nefrótico, glomerulonefritis membranosa, síndrome nefrótico familiar, glomerulonefritis membranoproliferativa I y II, glomerulonefritis proliferativa mesangial, glomerulonefritis crónica, nefritis tubulointerticial aguda, nefritis tubulointerticial

crónica, glomerulonefritis post-estreptococal aguda, (PSGN), pielonefritis, nefritis lupus, nefritis crónica, nefritis intersticial, y glomerulonefritis post-estreptococal), trastornos de vasos sanguíneos de los riñones (ej., infarto de riñon, enfermedad renal ateroembólica, necrosis cortical, nefroesclerosis maligna, trombosis de vena renal, subperfusión renal, retinopatía renal, isquemia-reperfusión renal, embolismo de arterias renales, y estenosis de arterias renales), trastornos renales que resultan a partir de enfermedad del tracto urinario (ej., pielonefritis, hidronefrosis, urolitiasis (litiasis renal, nefrolitiasis), nefropatia de reflujo, infecciones del tracto urinario, retención urinaria, y uropatía obstructiva unilateral aguda o crónica.)

Además, las composiciones del invento se puede utilizar para diagnosticar, pronosticar, prevenir, y/o tratar trastornos metabólicos y congénitos del riñón (ej., uremia, amiloidosis renal, ostodiostrofia renal, acidosis tubular renal, glicosuria renal, diabetes nefrogénica insipidus, cistinuria, síndrome de Fanconi, osteosis fibrocística renal (raquitis renal), enfermedad de Hartnup, síndrome de Bartter, síndrome de Liddle, de enfermedad renal policística, enfermedad cística medular, riñón de esponja medular, síndrome de Alport, síndrome de uña patelada, síndrome nefrótico congénito, síndrome de CRUSH, riñón de herradura, nefropatia diabética, diabetes nefrótica insipidus, nefropatia analgésicos, cálculos renales, y nefropatia membranosa), y trastornos autoinmunes del riñón (ej., lupus sistémico eritematoso (SLE), síndrome de Goodpasture, nefropatía IgA, y glomerulonefritis proliferativa mesangial IgM).

Las composiciones del invento también se puede utilizar para diagnosticar, pronosticar, prevenir, y/o tratar trastornos escleróticos necróticos del riñón (ej., glomeruloesclerosis, nefropatia diabética, glomeruloesclerosis segmental focal (FSGS), glomerulonefritis necrotizante, y necrosis papilar renal), cánceres del riñón (ej., nefroma, hipernefroma, nefroblastoma, cáncer celular renal, cáncer celular transicional, adenocarcinoma renal, cáncer celular escamoso, y tumor de Wilm), e imbalances electrolíticos (ej., nefrocalcinosis, piuria, edema, hidronefritis, proteinuria, hiponatremia, hipermatremia, hipocalemia, hipercalemia, hipofosfatemia, e hiperfosfatemia).

Las composiciones del invento se pueden administrar utilizando cualquier método conocido en el estado de la técnica, incluyendo, pero sin limitar a inyección de aguja directa, en el sitio de liberación, inyección intravenosa, administración tópica, infusión de catéter, inyectores biolísticos, aceleradores de partículas, depósitos de esponja *gelfoam*, otros materiales de depósito comercialmente disponibles, bombas osmóticas, fórmulaciones farmacéuticas sólidas orales o supositoriales, aplicaciones decantadoras o tópicas durante cirugía, liberación en aerosol. Tales métodos son conocidos en el estado la técnica. Las composiciones del invento se paran administrar como parte de un terapéutico, tal como se describe en detalle más adelante. Los métodos de liberación de polinucleotidos del invento son descritos en detalle en la presente.

Trastornos cardiovasculares

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se puede utilizar para tratar, prevenir, diagnosticar, y/o pronosticar trastornos cardiovasculares, incluyendo pero sin limitar a enfermedad arterial periférica, tal como isquemia de extremidades.

Los trastornos cardiovasculares incluyen, pero no están limitados a anormalidades cardiovasculares tales como fistula arterio-arterial, fistula arteriovenosa, malformaciones arteriovenosas cerebrales, defectos cardiacos congénitos, atresia pulmonar, y síndrome Scinmitar. Los defectos cardiacos congénitos incluyen, pero no están limitados a coartación aórtica, cor triatriatum, anormalidades de vasos coronarios, corazón en *crisscross*, dextrocardia, conducto arterioso patente, anomalía de Ebstein, complejo de Eisenmenger, síndrome cardíaco izquierdo

hipoplásico, tetralogía de Fallot, transposición de grandes vasos, ventrículo derecho de salida doble, atresia tricúspide, arteria trocal persistente, y defectos septales cardiacos, tal como defecto septal aortopulmonar, defectos de cojin endocardiaco, síndrome de Lutembacher, trilogía de Fallot, defectos septales cardiacos ventriculares.

Los trastornos cardiovasculares también incluyen, pero no están limitados a enfermedad cardiaca, tales como arritmias, enfermedad cardiaca carcinoide, salida cardiaca alta, salida cardiaca baja, taponamiento cardíaco, endocarditis (incluyendo bacteriana), aneurisma cardiaco, paro cardíaco, falla cardiaca congestiva, cardiomiopatía congestiva, dispnea paroximal, edema cardíaco, hipertrofia cardiaca, cardiomiopatía congestiva, hipertrofia ventricular izquierda, hipertrofia ventricular derecha, ruptura cardiaca post-infarto, ruptura septal ventricular, enfermedades de válvulas cardiacas, enfermedades del miocardio, isquemia de miocardio, efusión pericardial, pericarditis (incluyendo constructiva y tuberculosa), pneumopericardio, síndrome de postpericardiotomia, enfermedad cardiaca pulmonar, enfermedad cardiaca reumática, disfunción ventricular, hiperemia, complicaciones cardiovasculares por embarazo, síndrome de cimitarra, sífilis cardiovascular, y tuberculosis cardiovascular.

Las arritmias incluyen, pero no están limitadas a arritmia de seno, trepidación auricular, bradicardia, extrasístole, síndrome de Adams-Stokes, bloque de rama fascicular, bloque sinoauricular, síndrome QT largo, parasístole, síndrome de Lown-Ganong-Levine, síndrome de pre-excitación tipo Mahaim, síndrome Wolff-Parkinson-White, síndrome del seno enfermo, taquicardias, y fibrilación ventricular. Las taquicardias incluyen taquicardia paroximal, taquicardia supraventricular, ritmo yodoventricular acelerado, taquicardia de reentrada nodal atrioventricular, taquicardia atrial ectópica, taquicardia de unión ectópica, taquicardia de reentrada nodal sinoauricular, taquicardia del seno, Torsades de Pointes, y taquicardia ventricular.

Las enfermedades de válvulas cardiacas incluyen, pero no están limitadas a insuficiencia de válvulas aórticas, estenosis de válvulas aórticas, soplos cardíacos, prolapso de válvulas aórticas, prolapso de válvulas mitrales, prolapso de válvulas tricúspides, insuficiencia de válvulas mitrales, estenosis de válvulas mitrales, atresia pulmonar, insuficiencia de válvulas pulmonares, estenosis de válvulas pulmonares, atresia tricúspide, insuficiencia de válvulas tricúspides, y estenosis de válvulas tricúspides.

Las enfermedades del miocardio incluyen, pero no están limitadas a cardiomiopatía alcohólica, cardiomiopatía congestiva, cardiomiopatía hipertrófica, estenosis subvalvular aórtica, estenosis subvalvular pulmonar, cardiomiopatía restrictiva, cardiomiopatía de Chagas, fibroelastosis endocardial, fibrosis endomiocardial, síndrome de Kearns, lesión de reperfusión miocardial, y miocarditis.

Las isquemias del miocardio incluyen, pero no están limitadas a enfermedad coronaria, tal como angina de pecho, aneurisma coronario, arterioesclerosis coronaria, trombosis coronaria, vasoespasmo coronario, infección del miocardio, aturdimiento miocárdiaco.

Las enfermedades cardiovasculares también incluyen enfermedades vasculares tales como aneurismas, angiodisplasia, angiomatosis, angiomatosis bacilar, enfermedad de Hippel-Lindau, síndrome de Klippel-Trenaunay-Weber, Síndrome de Sturge-Weber, edema angioneurótico, enfermedades aórticas, Arteritis de Takayasu, aortitis, síndrome de Leriche, enfermedades oclusivas arteriales, arteritis, enarteritis, poliarteritis nodosa, trastornos cerebrovasculares, angiopatias diabéticas, retinopatía diabética, embolismos, trombosis, eritromelalgia, hemorroides, enfermedad veno-oclusiva hepática, hipotensión, isquemia, enfermedades vasculares periféricas, plebitis, enfermedad veno-oclusiva pulmonar, enfermedad de Raynaud, síndrome de CREST, oclusión de vena retinal, síndrome Scimitar, síndrome de vena cava superior, telangestasia, ataxia

telangestasia, telangestasia hemorrágica hereditaria, varicocele, venas varicosas, ulcera varicosa, vasculitis, e insuficiencia venosa.

Los aneurismas incluyen, pero no están limitados a aneurismas de disección, aneurismas falsos, aneurismas infectados, aneurismas rotos, aneurismas aórticos, aneurismas cerebrales, aneurismas coronarios, aneurismas cardiacos, y aneurismas iliacos.

Los trastornos oclusivos arteriales incluyen, pero no están limitados a arterioesclerosis, claudicación intermitente, estenosis carótida, displasias fibromusculares, oclusión vascular mesentérica, enfermedad de Moyamoya, obstrucción de la arteria renal, oclusión de la arteria retinal, tromboangiítis obliterante.

Los trastornos cerebrovasculares incluyen, pero no están limitados a enfermedades de la arteria carótida, angiopatia amiloide cerebral, aneurisma cerebral, anoxia cerebral, arterioesclerosis cerebral, malformación arteriovenosa cerebral, enfermedades de arterias cerebrales, embolismo cerebral y trombosis, trombosis de arterias carótidas, trombosis del seno, síndrome de Wallenberg, hemorragia cerebral, hematoma epidural, hematoma subdural, hemorragia subaraxnoide, infarto cerebral, isquemia cerebral, (incluyendo temporal), síndrome del robo de la subclavia, leucomalacia periventricular, cefalea vascular, cefalea acuminada, insuficiencia de vertebrobacilar.

Los embolismos incluyen, pero no están limitados a embolismos de aire, embolismos de fluido amniótico, embolismos de colesterol, síndrome del dedo azul, embolismos grasos, embolismos pulmonares, y tromboembolismos. Trombosis incluyen, pero no están limitadas a trombosis coronaria, trombosis de vena hepática, oclusión de vena retinal, trombosis de arteria carótida, trombosis del seno, síndrome de Wallenberg, y tromboflebitis.

Los trastornos isquémicos incluyen, pero no están limitados a isquemia cerebral, colitis isquémica, síndromes de compartimentos, síndrome de compartimento anterior, isquemia miocardial, lesiones de reperfusión, e isquemia de extremidades periféricas. Vasculitis incluye, pero no está limitada a aortitis, arteritis, síndrome de behcet, síndrome de Churg-Strauss, síndrome mococutáneo linfonodular, tromboangiítis obliterante, vasculitis de hipersensibilidad, púrpura de Schoenlein-Henoch, vasculitis cutánea alérgica, y granulomatosis de Wegener.

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden administrar utilizando cualquier método conocido en el estado de la técnica, incluyendo, pero sin limitar a inyección de aguja directa en el sitio de liberación, inyección intravenosa, administración tópica, infusión de catéter, inyectores biolísticos, aceleradores de partículas, depósitos de esponja *gelfoam*, otros materiales de depósito comercialmente disponibles, bombas osmóticas, fórmulaciones farmacéuticas sólidas orales o supositoriales, aplicaciones decantadoras o tópicas durante cirugía, liberación en aerosol. Tales métodos son conocidos en el estado de la técnica. Métodos para liberar polinucleotidos son descritos en detalle más adelante.

Trastornos respiratorios

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para tratar, prevenir, diagnosticar, y/o pronosticar enfermedades y/o trastornos del sistema respiratorio.

Las enfermedades y trastornos del sistema respiratorio incluyen, pero no están limitadas a vestibulitis nasal, rinitis no alérgica (ej., rinitis aguda, rinitis crónica, rinitis atrófica, rinitis vasomotora), pólipos nasales, y sinusitis, angiofibromas juveniles, cáncer de la nariz y papilomas juveniles, pólipos de cuerdas vocales, nódulos (nódulos de las cuerdas vocales), úlceras de contacto, parálisis de cuerdas vocales, laringoceles, faringitis (ej., viral y bacteriana), tonsilitis, celulitis tonsilar, absceso parafaríngeo, laringitis, laringoceles, y cánceres de garganta (ej., cáncer

de la nasofaringe, cáncer de amígdala, cáncer del laringe), cáncer pulmonar (ej., carcinoma celular escamoso, carcinoma de células pequeñas (célula en avena avenocarcinoma), carcinoma de células grandes, y adenocarcinoma), trastornos alérgicos (neumonía eosinofílica, neumonitis de hipersensibilidad (ej., alveolitis alérgica extrínseca, neumonitis intersticial alérgica, pneumoconiosis por polvo orgánico, aspergilosis broncopulmonar alérgica, asma, granulomatosis de Wegener (vasculitis granulomatosa), síndrome de Goodpasture)), neumonía (ej., neumonía bacteriana (ej., *Streptococcus pneumoniae* (neumonía pneumococcal), *Staphycoccus aurens* (neumonía estafilococal), neumonía bacteriana gram-negativa (producida por, ej., *Klebsiella* y *Pseudomonas spp.*), neumonia por *Mycoplásma pneumoniae*, neumonía por *Hemophilus influenzae*, *Leigionella pneumophila* (enfermedad de los Legionarios), y *Chlamydia psittaci* (Psittacosis)), y pneumonia viral (ej., influenza, viruela de pollo (varicela).

Enfermedades y trastornos adicionales del sistema respiratorio incluyen, pero no están limitadas a bronquiolitis, polio (poliomielitis), crup, infección viral sincitial respiratoria, paperas, eritema infeccioso(quinta enfermedad), exantema súbito, panencefalitis progresiva por rubeola, rubéola, y panencefalitis esclerosante subaguda), neumonía fungal (ej., histoplasmosis, cocidioidomicosis, blastomicosis, infecciones micóticas en personas con sistemas inmunes severamente deprimidos (ej., criptococosis, producida por *cryptococcus neoformans*; aspergilosis, producida por *Aspergillus spp;* candidiasis, producida por *Candida;* y mucormicosis)), *Pneumocistis carinni* (pneumocistis pneumonia), neumonías atípicas (ej., *Mycoplasma* y *Chlamydia* spp.), neumonía de infección oportunista, neumonía nosocomial, neumonitis química, y neumonía de aspiración, trastornos pleurales (ej., pleuresía, efusión pleural, y neumotórax (ej., neumotórax espontáneo simple, neumotórax espontáneo complicado, neumotórax de tensión)), enfermedades de vías aereas obstructivas (ej., asma, enfermedad pulmonar obstructiva crónica (COPD), enfisema, bronquitis crónica o aguda), enfermedades pulmonares ocupacionales (ej., silicosis, pulmón negro (neumoconiosis de trabajadores del carbón), asbestosis, beriliosis, asma ocupacional, bisinosis, y neumoconiosis benignas), enfermedad pulmonar infiltrativa (ej., fibrosis pulmonar (ej., alveolitis fibrosa, neumonía intersticial usual), fibrosis pulmonar yodopática, neumonía intersticial descamativa, neumonía intersticial linfoide, histiocitosis X (ej., enfermedad de Letterer-Siwe, enfermedad de Hand-Schuller-Christian, granuloma eosinofílico), hemosiderosis pulmonar yodopática, sarcoidosis y proteinosis alveolar pulmonar), síndrome de distrés respiratorio agudo (también llamado, ej., síndrome de distrés respiratorio del adulto), edema, embolismo pulmonar, bronquitis (ej., viral, bacteriana), bronquiectasis, atelectasis, absceso pulmonar (causado por ej., *Staphylococcus aurens o Legionella pneumophila*), y fibrosis cística.

Actividad Anti-Angiogenesis

El balance natural entre estimuladores endógenos e inhibidores de angiogénesis es uno en el cual predominan las influencias inhibitorias. Rastinejad *Cell* 56:345-355 (1989). En estas instancias raras en las cuales ocurre neovascularización bajo condiciones fisiológicas normales, tal como curación de heridas, regeneración de órganos, desarrollo embriónico, y procesos reproductivos femeninos, la angiogénesis es regulada de manera severa y delimitada de manera espacial y temporal. Bajo condiciones de angiogenesis patológica tal como aquella que caracteriza crecimiento tumoral sólido, estos controles regulatorios fallan. La angiogenesis no regulada se vuelve patológica y sostiene progresión de muchas enfermedades neoplásicas y no neoplásicas. Un numero de enfermedades serias son gobernadas por neovascularización anormal incluyendo crecimiento tumoral sólido y metastasis, artritis, algunos tipos de trastornos oculares, y psoriasis. Ver, ej., reseñas de Moses *Biotech.* 9:630-634 (1991); Folkman, *N. Engl. J. Med.* 333:1757-1763 (1995); Auerbach *J. Microvasc. Res. 29:401-411 (1985); Folkman,* Avances en

Investigación de Cáncer, eds. Klein y Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Optalmol.* 94:715-743 (1982), Folkman. Science 221:719-725 (1983). En un número de condiciones patológicas, el proceso de angiogenesis contribuye al estado patológico. Por ejemplo, se han acumulado datos sigificativos los cuales indican que el crecimiento de tumores sólidos depende de angiogénesis. Folkman y Klagsbrun, *Science* 235:442-447 (1987).

El presente invento proporciona tratamiento de enfermedades o trastornos asociados con neovascularización mediante administración de proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento. Las condiciones malignas y metastáticas que se pueden tratar con los polinucleotidos que polipéptidos, o agonistas o antagonistas del invento incluyen, pero no están limitadas a, malignidades, tumores sólidos, y cánceres descritos en la presente y de otra forma conocidos en el estado de la técnica (para una reseña de tales transtornos, ver Fishman, Medicine, 2d Ed. J. B. Lippincott Co. Philadelphia (1985)), Por lo tanto, el presente invento proporciona un método para tratar una enfermedad relacionada con angiogenesis y/o trastorno, que comprende la administración a un individuo en necesidad del mismo de una cantidad terapeuticamente efectiva de una proteina de fusión de albúmina del invento y/o polinucleotidos que codifican una proteína de fusión de albúmina del invento. Por ejemplo, los proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar en la variedad de métodos adicionales con el objeto de tratar terapeuticamente un cáncer o tumor. Cánceres que se pueden tratar con proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen, pero no están limitados a tumores sólidos, incluyendo cáncer de próstata, pulmonar, de mama, ovárico, estomacal, pancreatico, laríngeo, esofágico, testicular, hepático, de colon, de recto, de cervix, uterino, de endometrio, renal, de vejiga, tiroideo; tumores primarios y metástasis; melanonas; glioblastoma; sarcoma de Kaposi, leiomiosarcoma, cáncer pulmonar de células pequeñas; cáncer colorectal, malignidades avanzadas; tumores sanguíneos tales como leucemias. Por ejemplo, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser liberados de manera tópica, con el objeto de tratar cánceres tales como cáncer de piel, tumores de cabeza y cuello, tumores de mama, y sarcoma de Kaposi.

Dentro de aún otros aspectos, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para tratar formas superficiales de cáncer de vejiga mediante, por ejemplo, administración intravesical. Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden liberar directamente en el tumor, o cerca del sitio del tumor, a través de inyección o de catéter. Naturalmente, como lo apreciará alguien con experiencia común, el modo apropiado de administración variará según el cáncer a ser tratado. Otros modos de administración son discutidos en la presente.

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles para tratar otros trastornos, además de cánceres, los cuales involucran angiogénesis. Estos trastornos incluyen, pero no están limitadas a tumores benignos, por ejemplo hemangiomas, neuromas acústicos, neurofibromas, tracomas, y granulomas piogénicos; placas arterosclerótica, enfermedades angiogénicas oculares, por ejemplo, retinopatía diabética, retinopatía por premadurez, degeneración macular, rechazo de injerto corneal, glaucoma neovascular, fibroplasia retrolental, rubeosis, retinoblastoma, (crecimiento de vasos sanguineos anormal) del ojo; artritis reumatoide, psoriasis; curación retardada de heridas; endometriosis; vasculogénesis, granulaciones; cicatrices hipertróficas, (queloides); fracturas en no uniones; escleroderma; tracoma, adhesiones vasculares,

angiogénesis del miocardio; colaterales coronarios, colaterales cerebrales, malformaciones arteriovenosas, angiogénesis de extremidades isquémicas, síndrome de Osler-Webber; neovascularización de placas; telangestasia; articulaciones hemofílicas; angiofibroma; displasia fibromuscular, granulación de heridas, enfermedad de Crohn; y ateroesclerosis.

Por ejemplo, dentro de un aspecto del presente invento se proporcionan métodos para tratar queloides y cicatrices hipertróficas, comprendiendo el paso de administrar proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento a un queloide o cicatriz hipertrófica.

Dentro de una realización del presente invento las proteínas del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento son directamente inyectados en un queloide o cicatriz hipertrófica, con el objeto de prevenir la progresión de estas lesiones. Esta terapia es de valor particular en el tratamiento profilático de condiciones que se conoce resultan en el desarrollo de cicatrices hipertróficas o queloides (ejemplo, quemaduras), que preferiblemente se inicia después que la fase proliferativa ha tenido tiempo para progresar (aproximadamente 14 días después de la lesión inicial), pero antes del desarrollo del queloide o cicatriz hipertrófica. Como se anotó anteriormente, el presente invento también proporciona métodos para tratar enfermedades neovasculares de los ojos, incluyendo por ejemplo, neovascularización corneal, glaucoma neovascular, retinopatía diabética proliferativa, fibroplasia retrolental y degeneración macular.

Además, los trastornos oculares asociados con neovascularización que se pueden tratar con las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen, pero no están limitadas a glaucoma neovascular, retinopatía diabética, retinoblastoma, fibroplasia retrolental, uveítis, retinopatía por premadurez (degeneración macular), neovascularización de injerto corneal, así como también otras enfermedades inflamatorias oculares, enfermedades y tumores oculares asociados con neovascularización coroidea o del iris. Ver, ej., reseñas de Waltman, *Am. J. Ophthal. 85*:704-710 (1978) y Gartner *Surv. Ophthal. 22*:291-312 (1978).

Por lo tanto, dentro de un aspecto del presente invento se proporcionan métodos para tratar enfermedades neovasculares de los ojos tales como neovascularización corneana (incluyendo neovascularización de injerto corneal), comprendiendo el paso de administrar a un paciente una cantidad terapeuticamente efectiva de un compuesto (ejemplo, proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento) a la cornea, tal que la formación de vasos sanguíneos es inhibida. Brevemente, la cornea es un tejido que normalmente carece de vasos sanguíneos. En ciertas condiciones patológicas sin embargo, se pueden extender capilaridades hacia la cornea desde el plexo vascular del limbo. Cuando la cornea se vasculariza, también se nubla, produciendo una disminución en la agudeza visual del paciente. La pérdida visual puede ser completa si la cornea se opaca completamente. Una amplia variedad de trastornos pueden resultar en neovascularización corneal, incluyendo por ejemplo, infecciones corneales (ej., tracoma, queratitis del herpes simplex, leishmaniasis y oncocercosis), procesos inmunológicos (ej., rechazo de injertos y síndrome de Stevens-Johnson), quemaduras alcalinas, trauma, inflamación (de cualquier causa), estados de deficiencia tóxica y nutricional, y como una complicación por utilización de lentes de contacto.

Dentro de realizaciones particularmente preferidas del invento, las composiciones se pueden preparar para administración tópica en solución salina (combinadas con cualquiera de los preservativos y agentes antimicrobianos comúnmente utilizados en preparaciones oculares), y se pueden administrar en forma de gotas para ojos. La solución o suspensión se puede preparar en su forma pura y administrar varias veces al día. Alternativamente, las composiciones anti-

angiogénicas, preparadas como se describió anteriormente, también se pueden administrar directamente a la cornea. Dentro de realizaciones adicionales, los factores anti-angiogénicos o composiciones anti-angiogénicas se pueden utilizar como un aditamento a terapia esteroide convencional. La terapia tópica también puede ser profilacticamente útil en lesiones corneales las cuales son conocidas por tener una alta probabilidad de inducir una respuesta angiogénica (tal como quemaduras químicas). En estas instancias el tratamiento, probablemente en combinación con esteroides, puede ser sustituido inmediatamente para ayudar a prevenir complicaciones subsiguientes.

Dentro de otras realizaciones, los compuestos descritos anteriormente se pueden inyectar directamente en el estroma corneal por parte de un oftalmólogo bajo guia microscópica. El sitio preferido de inyección puede variar con la morfología de la lesión individual, pero la meta de la administración sería colocar la composición en el frente de avance de la vasculatura (es decir, esparcida entre los vasos sanguíneos y la cornea normal). En la mayoría de los casos esto involucraría inyección corneal perilímbica para "proteger" la cornea de los vasos sanguíneos progresivos. Este método también se puede utilizar poco después de un traumatismo corneal con el objeto de prevenir profilacticamente neovascularización corneal. En esta situación el material se podría inyectar en la cornea perilímbica esparcido entre la lesión corneal y su suministro sanguíneo límbico potencial indeseado. Tales métodos también se pueden utilizar en una forma similar para prevenir invasión capilar de corneas trasplantadas. En una forma de liberación sostenida, las inyecciones pueden solamente requerir 2-3 veces por año. También se podría agregar un asteroide a la solución de inyección para reducir inflamación que resulta de la inyección misma.

Dentro de otro aspecto del presente invento, se proporcionan métodos para tratar glaucoma neovascular, comprendiendo el paso de administrar a un paciente una cantidad terapeuticamente efectiva de una proteína de fusión de albúmina del invento y/o polinucleotidos que codifican una proteína de fusión de albúmina del invento al ojo, tal que la formación de vasos sanguíneos es inhibida. En una realización, el compuesto se puede administrar de manera tópica al ojo con el objeto de tratar formas tempranas de glaucoma neovascular. Dentro de otras realizaciones, el compuesto se puede implantar mediante inyección en la región del ángulo de cámara anterior. Dentro de otras realizaciones, el compuesto también se puede colocar en cualquier ubicación tal que el compuesto es continuamente liberado en el humor acuoso. Dentro de otro aspecto del presente invento, se proporcionan métodos para tratar retinopatía diabética proliferativa, comprendiendo el paso de administrar a un paciente en los ojos, una cantidad terapeuticamente efectiva de una proteína de fusión de albúmina del invento y/o polinucleotidos que codifican una proteína de fusión de albúmina del invento, tal que la formación de vasos sanguíneos es inhibida.

Dentro de realizaciones particularmente preferidas del invento, la retinopatía diabética proliferativa se puede tratar mediante inyección en el humor acuoso o el vitreo, con el objeto de aumentar la concentración local del polinucleotido, polipéptido, agonista y/o antagonista en la retina. Preferiblemente, este tratamiento se debe iniciar antes de la adquisición de enfermedad severa requiriendo fotocoagulación.

Dentro de otro aspecto del presente invento se proporcionan métodos para tratar fibroplasia retrolental, comprendiendo el paso de administrar a un paciente una cantidad terapeuticamente efectiva de una proteína de fusión de albúmina del invento y/o polinucleotidos que codifican una proteína de fusión de albúmina del invento en el ojo, tal que la formación de vasos sanguíneos es inhibida. El compuesto se puede administrar de manera tópica, a través de inyección intravenosa y/o a través de implantes intraoculares.

Adicionalmente, los trastornos que se pueden tratar con proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen, pero no están limitados a, hemangiomas, artritis, soriasis, angiofibroma, placas arterosclerótica, curación retardada de heridas, granulaciones, articulaciones hemofílicas, cicatrices hipertróficas, fracturas sin unión, síndrome de Osler-Weber, granuloma piogénico, escleroderma, tracoma, y adhesiones vasculares.

Además, los trastornos y/o condiciones, que se pueden tratar, prevenir, diagnosticar, y/o pronosticar con las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen, pero no están limitados a tumores sólidos, tumores sanguíneos tales como leucemias, metástasis tumoral, sarcoma de Kaposi, tumores benignos, por ejemplo hemangiomas, neuromas acústicos, neurofibromas, tracomas, y granulomas piogénicos, artritis reumatoide, soriasis, enfermedades angiogénicas oculares, por ejemplo, retinopatía diabética, retinopatía por premadurez (degeneración macular), rechazo de injertos corneales, glaucoma neovascular, fibroplasia retrolental, rubeosis, retinoblastoma, y uveítis, curación retardada de heridas, endometriosis, vasculogénesis, granulaciones, cicatrices hipertróficas (queloides), fracturas sin unión, escleroderma, tracoma, adhesiones vasculares, angiogénesis del miocardio, colaterales coronarios, colaterales cerebrales, malformaciones arteriovenosas, angiogénesis de extremidades isquémicas, síndrome de Osler-Webber, neovascularización de placas, telangestasia, articulaciones hemofílicas, angiofibroma, displasia fibromuscular, granulación de heridas, enfermedad de Crohn, ateroesclerosis, agente de control natal mediante prevención de neovascularización requerida para implantes de embriones controlando menstruación, enfermedades que tienen angiogénesis como una consecuencia patológica tal como la enfermedad del rasguño de gato (Rochele minalia quintosa), úlceras (Helicobacter pylori), fiebre de oroya y angiomatosis bacilar.

En un aspecto del método de control natal, una cantidad de compuesto suficiente para bloquear el implante del embrión es administrada antes o después que el coito y la fertilización hayan ocurrido, proporcionando así un método efectivo de control natal, posiblemente un método de la "mañana siguiente". Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento también se pueden utilizar para controlar menstruación o se pueden administrar ya sea como un fluido de lavado peritoneal o para implante peritoneal en el tratamiento de endometriosis.

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden incorporar en suturas quirúrgicas con el objeto de prevenir granulomas por suturas.

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar en una amplia variedad de procedimientos quirúrgicos. Por ejemplo, dentro de un aspecto del presente invento, una composición (en la forma de, por ejemplo, un espray o película) se puede utilizar para recubrir un área antes de retirar un tumor, con el objeto de aislar tejidos circundantes normales de tejidos malignos y/o prevenir la diseminación de la enfermedad hacia tejidos circundantes. Dentro de otros aspectos del presente invento, las composiciones (ej., en la forma de un espray) se pueden liberar a través de procedimientos endoscópicos con el objeto de recubrir tumores, o inhibir angiogénesis en un sitio deseado. Dentro de aún otros aspectos del presente invento, las mallas quirúrgicas que han sido recubiertas con composiciones anti-angiogénicas del presente invento se puede utilizar en cualquier procedimiento en donde una malla quirúrgica puede ser utilizada. Por ejemplo, dentro de una realización del invento una malla quirúrgica cargada con una composición anti angiogénica se puede utilizar durante cirugía de resección de cáncer abdominal (ej.,

subsecuente a resección del colon) con el objeto de proporcionar soporte a la estructura, y para liberar una cantidad del factor anti-angiogénico.

Dentro de aspectos adicionales del presente invento, se proporcionan métodos para tratar sitios de excisión tumoral, comprendiendo administración de proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento en las márgenes de recesión de un tumor luego de excisión, tal que la recurrencia local de cáncer y la formación de nuevos vasos sanguíneos en el sitio es inhibida. Dentro de una realización del invento, el compuesto anti-angiogénico se administra directamente en el sitio de excisión tumoral (ej., aplicado mediante frotación, rozamiento o de otra forma recubriendo las margenes de resección del tumor con el compuesto anti-angiogénico). Alternativamente, los compuestos anti-angiogénicos se pueden incorporar en pastas quirúrgicas conocidas antes de administración. Dentro de realizaciones particularmente preferidas del invento, los compuestos anti-angiogénicos son aplicados después de recesiones hepáticas para malignidad, y después de operaciones neuroquirúrgicas.

Dentro de un aspecto presente invento, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden administrar a la margen de recesión de una amplia variedad de tumores, incluyendo por ejemplo, tumores de mama, colon, cerebrales y hepáticos. Por ejemplo, dentro de una realización del invento, los compuestos anti-angiogénicos se pueden administrar en el sitio de un tumor neurológico luego de excisión, tal que la formación de nuevos vasos sanguíneos en el sitio es inhibida.

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser administradas junto con otros factores anti-angiogénicos. Ejemplos representativos de factores anti-angiogénicos incluyen: Factor anti-invasivo, ácido retinóico y derivados del mismo, paclitaxel, Suramina, Inhibidor de Tejidos de Metaloproteinasa-1, inhibidor de tejidos de metaloproteinasa-2, Inhibidor del Activador del Plasminógeno tipo 1, Inhibidor del Activador del Plasminógeno tipo 2, y varias formas de los metales de transición más livianos del "grupo-d".

Los metales de transición más livianos del "grupo d" incluyen, por ejemplo, vanadio, molibdeno, tugsteno, titanio, niobio, y especies de tantalo. Tales especies metálicas de transición pueden formar complejos metálicos de transición.

Ejemplos representativos de complejos de vanadio incluyen complejos de oxo vanadio tales como complejos de vanadato y vanadilo. Complejos de vanadato apropiados incluyen complejos de metavanadato y ortovanadato tales como, por ejemplo, acetilacetona de vanadilo y sulfato de vanadilo incluyendo hidratos de sulfato de vanadilo tales como mono y trihidratos de sulfato de vanadilo.

Ejemplos representativos de complejos de tugsteno y molibdeno también incluyen oxo complejos. Complejos de oxo tugsteno apropiados incluyen complejos de tugsteno y complejos de óxido de tugsteno. Complejos de tugsteno apropiados incluyen tugsteno de amonio, tugsteno de calcio, dihidrato de tugsteno de sodio, y ácido tugstíco. Óxidos de tugsteno apropiados incluyen óxido de tugsteno (IV) y óxido de tugsteno (VI). Complejos de oxo molibdeno apropiados incluyen molibdato, óxido de molibdeno, y complejos de molibdenilo. Complejos de molibdato apropiados incluyen molibdato de amonio y sus hidratos, molibdato de sodio y sus hidratos, molibdato de potasio y sus hidratos. Óxidos de molibdeno apropiados incluyen óxido de molibdeno (IV), óxido de molibdeno (VI), y ácido molíbdico. Complejos de molibdeno apropiados incluyen, por ejemplo, acetilacetonato de molibdenilo. Otros complejos de tugsteno y molibdeno apropiados incluyen derivados de hidroxo derivados de, por ejemplo, glicerol, ácido tartárico, y azúcares.

Una amplia variedad de otros factores anti-angiogénicos también se pueden utilizar dentro del contexto del presente invento. Ejemplos representativos incluyen factor plaquetario 4; sulfato de protamina; derivados de quitina sulfatada (preparados a partir de cascarones de jaiba reina), (Murata, Cancer Res. 51:22-26, 1991); Complejo Polisacárido Sulfatado - peptidoglicano (SP-PG) (la función de este compuesto puede ser mejorada mediante la presencia de esteroides tales como estrógeno, y citrato de tamoxifen); Estaurosporina; moduladores de metabolismo de matriz, incluyendo por ejemplo, análogos de prolina, cishidroxiprolina, d, L-3,4-dehidroprolina, tiaprolina, alfa,alfa-dipiridilo, fumarato de aminopropionitrilo; 4-propil-5-(4-piridinil)-2(3H)-oxazolona; Metotrexato; Mitoxantrona; Heparina; Interferones; 2 macroglobulina sérica; ChIMP-3 (Pavloff, J. Bio. Chem. 267:17321-17326, (1992)); Quimostatina (Tomkinson, Biochem J. 286:475-480, (1992)); Tetradecasulfato de Ciclodextrina; Eponemicina; Camptotecina; Fumagillina (Ingber, Nature 348:555-557, 1990); Tiomalato de Sodio de Oro ("GST"; Matsubara y Ziff, J. Clin. Invest. 79:1440-1446, (1987)); anticolagenasa-suero; alfa2-antiplasmina (Holmes J. Biol. Chem. 262(4):1659-1664, (1987); Bisantreno (Instituto Nacional de Cáncer); Lobenzarit disódico (ácido N-(2)-carboxifenil-4-cloroantronílico disódico o "CCA", Takeuchi, Agents Actions 36:312-316, (1992)); Talidomida; esteroide angostático; AGM-1470; carboxinaminolmidazol; e inhibidores de mataloproteinasa tales como BB94.

Trastornos a nivel celular

Las enfermedades asociadas con supervivencia aumentada de células o la inhibición de apoptosis que podrían ser tratadas, previas, diagnosticados, y/o pronosticados utilizando proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, incluyen cánceres (tales como linfomas foliculares, carcinomas con mutaciones p53, y tumores dependientes de hormonas, incluyendo, pero sin limitar a cáncer de colon, tumores cardiacos, cáncer pancreático, melanoma, retinoblastoma, glioblastoma, cáncer pulmonar, cáncer intestinal, cáncer testicular, cáncer estomacal, neuroblastoma, mixoma, mioma, linfoma, endotelioma, osteoblastoma, osteoclastoma, osteosarcoma, condrosarcoma, adenoma, cáncer de mama, cáncer de próstata, sarcoma de Kaposi y cáncer ovárico); trastornos autoinmunes (tales como, esclerosis múltiple, síndrome de Sjogren, tiroiditis de Hashimoto, cirrosis biliar, enfermedad de Bencet, enfermedad de Crohn, polimiositis, lupus sistémico eritematoso y glomerulonefritis inmune relacionada y artritis reumatoide) e infecciones virales (tales como virus del herpes, poxvirus y adenovirus), inflamación, enfermedad de injerto vs. huésped, rechazo agudo de injertos, rechazo crónico de injertos.

En realizaciones preferidas, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento son utilizados para inhibir crecimiento, progresión, y/o metástasis de cánceres, en particular aquellos listados anteriormente.

Condiciones o enfermedades adicionales asociadas con supervivencia aumentada de células que podrían ser tratadas o detectadas mediante proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen, pero no están limitadas a, progresión, y/o metástasis de malignidades y trastornos relacionados tales como leucemia (incluyendo leucemias agudas, leucemia mielocitica aguda (incluyendo mieloblástica, promielocítica, mielomonocítica, monocítica, y eritroleucemia)) y leucemias crónicas (ej., leucemia mielocitica crónica (granulocítica) y leucemia linfocítica crónica)), policitemia vera, linfomas (ej., enfermedad de Hodgkin y enfermedad no Hodgkin), mieloma múltiple, macroglobulinemia de Waldenstrom, enfermedad de cadena pesada, y tumores sólidos incluyendo, pero sin limitar a sarcomas y carcinomas tales como fibrosarcoma, mixosarcoma, liposarcoma, condrosarcoma, sarcoma osteogénico, cordoma, angiosarcoma, endoteliosarcoma,

linfangiosarcoma, linfangioendoteliosarcoma, sinovioma, mesotelioma, tumor de Ewing, leimiosarcoma, rabdomiosarcoma, carcinoma de colon, cáncer pancreatico, cáncer de mama, cáncer ovárico, cáncer de próstata, carcinoma celular escamoso, carcinoma de células basales, adenocarcinoma, carcinoma de glándulas sudoríparas, carcinoma de glándulas sebaceas, carcinoma papilar, adenocarcinomas papilares, cistadenocarcinoma, carcinoma medular, carcinoma broncogénico, carcinoma de células renales, hematoma, carcinoma de ducto biliar, coriocarcinoma, seminoma, carcinoma embrional, tumor de Wilm, cáncer cervical, tumor testicular, carcinoma pulmonar, carcinoma pulmonar de células pequeñas, carcinoma de vejiga, carcinoma epitelial, glioma, astrocitoma, meduloblastoma, craneofaringioma, ependimoma, pinealoma, emangioblastoma, neuroma acústico, oligodendroglioma, menangioma, melanoma, neuroblastoma, y retinoblastoma.

Enfermedades asociadas con apoptosis aumentada que podrían ser tratadas, prevenidas, diagnosticadas, y/o pronosticadas utilizando proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, incluyen, pero no están limitadas a SIDA; trastornos neurodegenerativos (tals como enfermedad de Alzheimer, enfermedad de Parkinson, esclerosis lateral amiotrófica, rinitis pigmentosa, degeneración cerebelar, y tumor cerebral o enfermedad asociada); trastornos autoinmunes (tales como, esclerosis múltiple, síndrome de Sjogren, tiroiditis de Hashimoto, cirrosis biliar, enfermedad de Behcet, enfermedad de Crohn, polimiositis, lupus sistémico eritematoso y glomerulonefritis inmune relacionada y artritis reumatoide) síndromes mielodisplásicos (tales como anemia aplástica), enfermedad de injerto versus huesped, lesión isquémica (tal como aquella causa mediante infarto del miocardio, apoplejía y lesión de reperfusión), lesión hepática (ej., lesión hepática relacionada con hepatitis, lesión de isquemia /reperfusión, colestosis (lesión de ducto biliar) y cáncer hepático); enfermedad hepática inducida toxinas (tal como aquella causa por alcohol), choque séptico, cachexia y anorexia.

Curación de Heridas y Proliferación Celular Epitelial

Según un aspecto adicional del presente invento, se proporciona un proceso para utilizar proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, para propósitos terapéuticos, por ejemplo, para estimular proliferación de células epiteliales y queratinocitos basales para propósitos de curación de heridas, y para estimular producción de folículos capilares y curación de heridas dérmicas. Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, pueden ser clínicamente útiles para estimular curación de heridas incluyendo heridas quirúrgicas, heridas excisionales, heridas profundas involucrando daños de dermis y epidermis, heridas de tejido ocular, heridas de tejido dental, heridas de cavidad oral, úlceras diabéticas, úlceras dérmicas, úlceras de decubito, úlceras arteriales, úlceras de estasia venosa, quemaduras que resultan de exposición al calor o químicos, y otras condiciones de curación de heridas anormales tales como uremia, desnutrición, deficiencias de vitaminas y complicaciones asociadas con tratamiento sistémico con esteroides, terapia de radiación y fármacos antineoplásicos y antimetabolitos. Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se pueden utilizar para promover restablecimiento dérmico subsiguiente a pérdida dérmica.

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se pueden utilizar para aumentar la adherencia de injertos de piel en un lecho de herida y para estimular re-epitelialización a partir del lecho de herida. Los siguientes son tipos de injertos en los cuales las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se pueden utilizar para

aumentar adherencia a un lecho de herida: autoinjertos, aloinjertos, injerto autodérmico, injertos autoepdérmicos, injertos avaculares, injertos Blair-Brown, injerto óseo, injertos brefoplásicos, injerto de cutis, injerto retardado, injerto dérmico, injerto epidérmico, injerto de fascia, injerto de espesor completo, injerto heterólogo, xenoinjerto, injerto homologo, injerto hiperplásico, injerto laminar, injerto en malla, injerto mucosal, injerto de Ollier-Thiersch, injerto de omental, injerto de parche, injerto de pedículo, injerto de penetración, injerto de espesor dividido, injerto de espesor parcial. Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se pueden utilizar para promover resistencia dérmica y/o mejorar la apariencia de piel envejecida.

Se cree que las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, también producirán cambios en proliferación de hepatocitos, y proliferación celular epitelial en el pulmón, mama, páncreas, estómago, intestino delgado e intestino grueso. Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, pueden promover la proliferación de células epiteliales tales como sebocitos, folículos pilosos, hepatocitos, monocitos tipo II, células calciformes productoras de moco, y otras células epiteliales y sus progenitores contenidos dentro de la piel, pulmón, hígado, y tracto gastrointestinal. Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, pueden promover proliferación de células endoteliales, queratinocitos, y queratinocitos basales.

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, también se pueden utilizar para reducir los efectos secundarios de toxicidad intestinal que resultan a partir de tratamientos de radiación, quimioterapia o infecciones virales. Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, pueden tener un efecto citoprotector sobre la mucosa del intestino delgado. Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, pueden también estimular curación de mucositis (úlceras en la boca) que resulta a partir de quimioterapia e infecciones virales.

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, podrían además ser utilizados en regeneración completa de piel en defectos dérmicos de espesor total y parcial, incluyendo quemaduras, (es decir, repoblación de folículos pilosos, glándulas sudoríparas, y glándulas sebaceas), tratamiento de otros defectos dérmicos tales como psoriasis. Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se pueden utilizar para tratar epidermólisis bulosa, un defecto de adherencia de la epidermis a la dermis subyacente el cual resulta en ampollas frecuentes abiertas y dolorosas mediante reepitelialización acelerada de estas lesiones. Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, también se pueden utilizar para tratar úlceras gástricas y duodenales y ayudar a curar mediante formación de cicatrices del revestimiento mucosal y regeneración de la mucosa glandular y revestimiento mucosal duodenal de manera más rápida. Enfermedades intestinales inflamatorias, tal como la enfermedad de Crohn y colitis ulcerativa, son enfermedades que resultan en la destrucción de la superficie mucosal del intestino delgado o grueso, respectivamente. Por lo tanto, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, podrían ser utilizados para promover el reestablecimiento de la superficie mucosal para ayudar en la curación rápida y para prevenir la progresión de la enfermedad intestinal inflamatoria. El tratamiento con proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del

invento, se espera tenga un efecto significativo sobre la producción de moco en todo el tracto gastrointestinal y pueda ser utilizado para proteger la mucosa intestinal de sustancias perjudiciales que son ingeridas o después de cirugía. Las proteínas de fusión de albúmina y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se pueden utilizar para tratar enfermedades asociadas con subexpresión.

Además, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se pueden utilizar para prevenir y curar daño en los pulmones debido a varios estados patológicos. Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, los cuales pueden estimular proliferación y diferenciación y promueven la reparación de alveolos y epitelio bronquial para prevenir y tratar daño pulmonar agudo o crónico. Por ejemplo, enfisema, el cual resulta en la pérdida progresiva de alveolos, y lesiones de inhalación, es decir, que resulta a partir de inhalación de humo y quemaduras, que producen necrosis del epitelio broquial y alveolos se puede tratar de manera efectiva utilizando los polinucleotidos o polipéptidos, agonistas o antagonistas del presente invento. También las proteínas de fusión de invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se podrían utilizar para estimular la proliferación y diferenciación de neumocitos tipo II, lo cual puede ayudar a tratar o prevenir enfermedad tal como enfermedades de membrana de hialina, tal como síndrome de distrés respiratorio infantil y displasia broncopulmonar, en bebes prematuros.

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, podrían estimular la proliferación y diferenciación de hepatocitos y, por lo tanto, se podrían utilizar para aliviar o tratar enfermedades y patologías hepáticas tal como falla hepática fulminante causada por cirrosis, daño hepático causado por hepatitis viral y sustancias tóxicas (es decir, acetaminofen, tetraholoruro de carbono y otras hepatotoxinas conocidas en el estado de la técnica).

Además, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se podrían utilizar para tratar o prevenir el comienzo de diabetes mellitus. En pacientes con diabetes tipos I y II recién diagnosticadas, donde alguna función de células de isletas permanece, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se podrían utilizar para mantener la función de isletas para aliviar, retardar o prevenir manifestación permanente de la enfermedad. También, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se pueden utilizar como un auxiliar en trasplante de células de isletas para mejorar o promover la función de células de isletas.

Actividad neural y enfermedades neurológicas

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para diagnosis y/o tratamiento de enfermedades, trastornos, daño o lesión del cerebro y/o sistema nervioso. Los trastornos del sistema nervioso que se pueden tratar con las composiciones del invento (ejemplo, proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento), incluyen, pero no están limitados a lesiones del sistema nervioso, y enfermedades o trastornos que resultan en ya sea una desconexión de axonas, una disminución o degeneración de neuronas, o desmielinación. Las lesiones del sistema nervioso que se pueden tratar en un paciente (incluyendo pacientes mamíferos humanos y no humanos) según los métodos del invento, incluyen pero no están limitadas a, las siguientes lesiones ya sea del sistema nervioso central (incluyendo médula espinal, cerebro) o sistema nervioso periférico: (1) lesiones isquémicas, en las cuales la falta de oxigeno en una porción del sistema nervioso resulta en muerte o lesión

neuronal, incluyendo isquemia o infarto cerebral, o isquemia o infarto de médula espinal; (2) lesiones traumáticas, incluyendo lesiones causadas por lesión física o asociada con cirugia, por ejemplo, lesiones que dividen una parte del sistema nervioso, o lesiones de compresión; (3) lesiones malignas, en las cuales una parte del sistema nervioso es destruido o lesionado por tejido maligno el cual es una malignidad asociada al sistema nervioso o una malignidad derivada de tejido diferente al sistema nervioso; (4) lesiones infecciosas, en las cuales una parte del sistema nervioso es destruido o lesionado como resultado de infección, por ejemplo, mediante un absceso o asociado con infección por virus de inmunodeficiencia humana, virus herpes zoster, o herpes simplex o con enfermedad de Lima, tuberculosis, o sifilis, (5) lesiones degenerativas, en las cuales una parte del sistema nervioso es destruido o lesionado como resultado de un proceso degenerativo incluyendo pero sin limitar a, degeneración asociada con enfermedad de Parkinson, enfermedad de Alzheimer, corea de Huntington, o esclerosis lateral amiotrófica (ALS); (6) lesiones asociadas con trastornos o enfermedades nutricionales, en los cuales una porción de sistema nervioso es destruido o lesionado nutricional o desorden de metabolismo incluyendo, pero sin limitar a deficiencia de vitamina B12, deficiencia de ácido fólico, enfermedad de Wernicke, ambliopía de tabaco-alcohol, enfermedad de Marchiafava-Bignami (degeneración primaria del cuerpo calloso), y degeneración cerebelar alcohólica; (7) lesiones neurológicas asociadas con enfermedades sistémicas incluyendo, pero sin limitar a diabetes (neuropatía diabética, perlesía de Bell), lupus sistémico eritematoso, o sarcoidosis; (8) lesiones producidas por sustancias tóxicas incluyendo alcohol, plomo, o neurotoxinas particulares; y (9) lesiones desmielinadas en las cuales una porción del sistema nervioso es destruido o lesionado por una enfermedad desmielinizante incluyendo, pero sin limitar a esclerosis múltiple, mielopatía asociada con virus de inmunodeficiencia humana, mielopatía transversa o varias etiologías, leucoencefalopatía multifocal progresiva, y mielinolisis de pontina central.

En una realización, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan para proteger células neurales de los efectos dañinos de hipoxia. En una realización preferida adicional, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan para proteger células neurales de efectos dañinos de hipoxia cerebral. Según esta realización, las composiciones del invento se utilizan para tratar o prevenir lesión celular neural asociada con hipoxia cerebral. En un aspecto no exclusivo de esta realización, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se utilizan para tratar o prevenir lesión celular neural asociada con isquemia cerebral. En otro aspecto no exclusivo de esta realización, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan para tratar o prevenir lesión celular neural asociada con infarto cerebral.

En otra realización preferida, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan para tratar o prevenir lesión celular neural asociada con apoplejía. En una realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan para tratar o prevenir lesión celular neural cerebral asociada con apoplejía.

En otra realización preferida, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan para tratar o prevenir lesión celular neural asociada con ataque cardíaco. En una realización específica, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión

de albúmina del invento se utilizan para tratar o prevenir lesión celular neural cerebral asociada con ataque cardíaco.

Las composiciones del invento que son útiles para tratar o prevenir un trastorno del sistema nervioso se pueden seleccionar al probar la actividad biológica en promoción de supervivencia o diferenciación de neuronas. Por ejemplo, y no a manera de limitación, las composiciones del invento que producen cualquiera de los siguientes efectos pueden ser útiles según el invento: (1) tiempo de supervivencia aumentado de neuronas en cultivo ya sea en presencia o ausencia de hipoxia o condiciones hipóxicas; (2) brote neural aumentado en cultivo o *in vivo*; (3) producción aumentada de una molécula asociada a neurona en cultivo o *in vivo, ej.*, acetiltransferasa de colina o acetilcolinesterasa con respecto a neuronas motoras; o (4) síntomas disminuidos de disfunción de neuronas *in vivo*. Tales efectos se pueden medir mediante cualquier método conocido en el estado de la técnica. En realizaciones preferidas no limitantes, la superviviencia aumentada de neuronas se puede medir de manera rutinaria utilizando un método establecido en la presente o de otra manera conocido en el estado de la técnica, tal como, por ejemplo, en Zhang, *Proc Natl Acad Sci USA 97*:3637-42 (2000) o en Arakawa, *J. Neurosci.* 10:3507-15 (1990); el brote neuronal aumentado se puede detectar mediante métodos conocidos en el estado de la técnica, tal como, por ejemplo, los métodos divulgados en Pestronk, *Exp. Neurol.*, 70:65-82 (1980), o Brown *Ann. Rev. Neurosci.*, 4:*17-42 (1981);* la producción aumentada de moléculas asociadas a neuronas se puede medir mediante bioensayo, ensayo enzimático, unión de anticuerpos, ensayo Northem blot, etc., utilizando técnicas conocidas en el estado de la técnica y dependiendo de la molecula a ser medida; y la disfunción de neuronas motoras se pueden medir al evaluar la manifestación física de trastorno de neuronas motoras, ej., debilidad, velocidad de conducción de neuronas motoras, o discapacidad funcional.

En realizaciones específicas, los trastornos de neuronas motoras que se pueden tratar según el invento incluyen, pero no están limitados a trastornos tales como infarto, infección, exposición a toxina, trauma, daño quirurgico, enfermedad degenerativa o malignidad que pueden afectar neuronas motoras así como también otros componentes del sistema nervioso, así como también trastornos que afectan selectivamente neuronas tales como esclerosis lateral amiotrófica, e incluyendo, pero sin limitar a atrofia muscular espinal progresiva, perlesía bulbar progresiva, esclerosis lateral primaria, atrofia muscular infantil y juvenil, parálisis bulbar progresiva infantil (síndrome de Fazio-Londe), poliomielitis y el síndrome post polio, y Neuropatía Motosensorial Hereditaria (Enfermedad de Charcot-Marie-Tooth).

Además, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden jugar un papel en supervivencia neuronal, formación de sinapsa, conductancia; diferenciación neural, etc. Por lo tanto, las composiciones del invento (incluyendo proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento) se pueden utilizar para diagnosticar y/o tratar o prevenir enfermedades o trastornos asociados con estos papeles, incluyendo, pero sin limitar a trastornos de aprendizaje y/o conocimiento. Las composiciones del invento también pueden ser útiles en el tratamiento o prevención de estados patológicos neurodegenerativos y/o trastornos de conducta. Tales estados patológicos neurodegenerativos y/o trastornos de conducta incluyen, pero no están limitados a enfermedad de Alzheimer, enfermedad de Parkinson, enfermedad de Huntington, síndrome de Tourette, esquizofrenia, manía, demencia, paranoia, trastorno compulsivo obsesivo, trastorno de pánico, discapacidades de aprendizaje, ALS, psicosis, autismo, y conductas alteradas, incluyendo trastornos en alimentación, patrones de sueño, balance, y percepción. Además, las composiciones del invento pueden jugar un rol en el tratamiento, prevención y/o detección de trastornos de desarrollo asociados con el desarrollo de embriones, o trastornos sexualmente ligados.

Adicionalmente, las proteínas de fusión de invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, pueden ser útiles para proteger células neurales de enfermedades, daño, trastornos, o lesión, asociados con trastornos cerebrovasculares incluyendo, pero sin limitar a enfermedades de la arteria carótida (ej., trombosis de la arteria carótida, estenosis carótida, o enfermedad de Moyamoya), angiopatía amiloide cerebral, aneurisma cerebral, anoxia cerebral, arterioesclerosis cerebral, malformaciones arteriovenosas cerebrales, enfermedades de arterias cerebrales, embolismo cerebral y trombosis (ej., trombosis de la arteria carótida, trombosis del seno, o síndrome de Wallenberg), hemorragia cerebral (ej., hematoma epidural o subdural, o hemorragia subaracnoide), infarto cerebral, isquemia cerebral (ej., isquemia cerebral temporal, síndrome del robo de la subclavia, o insuficiencia vertebrobasilar), demencia vascular (ej., multi-infarto), leucomalacia, y cefalea periventricular y vascular (cefalea acuminada o migrañas).

Según un aspecto aún adicional del presente invento, se proporciona un proceso para utilizar proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, por propósitos terapéuticos, por ejemplo, para estimular proliferación y/o diferenciación celular neurológica y/o diferenciación. Por lo tanto, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para tratar y/o detectar enfermedades neurológicas. Además, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se pueden utilizar como un marcador o detector de un trastorno o enfermedad particular del sistema nervioso.

Ejemplos de enfermedades neurológicas que se puede tratar o detectar con proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen, enfermedades cerebrales, tal como enfermedades cerebrales metabólicas las cuales incluyen fenilcetonuria tal como fenilcetonuria maternal, deficiencia de carboxilasa de pivurato, deficiencia de complejo de deshidrogenasa de pivurato, encefalopatía de Wernicke, edema cerebral, neoplasmas cerebrales tales como neoplasmas cerebelares los cuales incluyen neoplasmas infratentoriales, neoplasmas de ventrículo cerebral tal como neoplasmas de plexo coroide, neoplasmas hipotalámicos, neoplasmas supratentoriales, enfermedades cerebelares las cuales incluyen degeneración espinocerebelar tal como ataxia telangestasia, dissinergia cerebelar, ataxia de Friederich, enfermedad de Machado-Joseph, atrofia olivopontocerebelar, neoplasmas cerebelares tales como neoplasmas infratentoriales, esclerosis cerebral difusa tal como periaxialis de encefalitis, leucodistrofia de células globoides, leucodistrofia metacromática y panencefalitis esclerosante subaguda.

Enfermedades neurológicas adicionales que se pueden tratar o detectar con proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen trastornos cerebrovasculares (tales como enfermedades de la arteria carótida las cuales incluyen trombosis de la arteria carótida, estenosis carótida y enfermedad de Moyamoya), angiopatía amiloide cerebral, aneurisma cerebral, anoxia cerebral, arterioesclerosis cerebral, malformaciones arteriovenosas cerebrales, enfermedades de arterias cerebrales, embolismo cerebral y trombosis tal como trombosis de la arteria carótida, trombosis del seno y síndrome de Wallenberg, hemorragia cerebral tal como hematoma epidural, hematoma subdural y hemorragia subaracnoide, infarto cerebral, isquemia cerebral tal como isquemia cerebral temporal, síndrome subclaviano steal e insuficiencia vertebrobasilar, demencia vascular tal como demencia de multi-infarto, leucomalacia periventricular, cefalea vascular tal como cefalea de cluster y migraña.

Enfermedades neurológicas adicionales que se pueden tratar o detectar con proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento

incluyen demencia tal como complejo de demencia de SIDA, demencia presenil tal como enfermedad de Alzheimer y síndrome de Creutzfeldt-Jakob, demencia senil tal como enfermedad de Alzheimer y perlesía supranuclear progresiva, demencia vascular tal como demencia de multi-infarto, encefalitis la cual incluye encefalitis periaxialis, encefalitis viral tal como encefalitis epidémica, encefalitis japonesa, encefalitis de St. Louis, encefalitis tick-borne y Fibre del Nilo Occidental, encefalitis diseminada aguda, meningoencefalitis tal como síndrome uveomeningoencefálico, enfermedad de Parkinson post encefalítica y panencefalitis esclerosante subaguda, encefalomalacia tal como leucomalacia periventricular, epilepsia tal como epilepsia generalizada la cual incluye espasmos infantiles, epilepsia de ausencia, epilepsia mioclónica la cual incluye síndrome de MERRF, epilepsia tónica-clónica, epilepsia parcial tal como epilepsia parcial compleja, epilepsia de lóbulo frontal y epilepsia de lóbulo temporal, epilepsia post-traumática, condición epiléctica tal como Epilepsia Parcialis Continua, y síndrome de Hallervonder-Spatz.

Enfermedades neurológicas adicionales que se pueden tratar o detectar con proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen hidrocéfalo tal como síndrome de Dandy-Walker e hidrocéfalo de presión normal, enfermedades hipotalámicas tales como neoplasmas hipotalámicos, malaria cerebral, narcolepsia la cual incluye cataplexia, poliomelitis bulbar, seudotumor cerebral, síndrome de Rett, Síndrome de Reyes, enfermedades talámicas, toxoplasmosis cerebral, tuberculosa más intracraneal y síndrome de Zellweger, infecciones del sistema nervioso central tal como complejo de demencia por SIDA, acceso cerebral, empiema subdural, encefalomielitis tal como encefalomielitis equina, encefalomielitis equina venezolana, encefalomielitis hemorrágica necrotizante, visna, y malaria cerebral.

Enfermedades neurológicas adicionales que se pueden tratar o detectar con proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen meningitis tal como aracnoiditis, meningitis aséptica tal como meningitis viral la cual incluye coriomeningitis linfocítica, meningitis bacteriana la cual incluye Haemophilus Meningitis, Meningitis Listeria, Meningitis Meningococal tal como síndrome de Waterhouse-Friderichsen, Meningitis Neumococal y tuberculosis meningeal tal como meningitis criptococal, efusión subdural, meningoencefalitis tal como síndrome uvemeningoencefalítico, mielitis tal como mielitis transversa, neurosífilis tal como tabes dorsal, poliomelitis la cual incluye poliomelitis bulbar y síndrome de postpoliomelitis, enfermedades por prion (tal como síndrome de Creutzfeldt-Jakob, Encefalopatía Espongiforme Bovina, Síndrome de Gerstmann-Straussler, Kuru, Prurito Lumbar), y toxoplasmosis cerebral.

Enfermedades neurológicas adicionales que se pueden tratar o detectar con proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen neoplasmas del sistema nervioso central tales como neoplasmas cerebrales que incluyen neoplasmas cerebelares tales como neoplasmas infratentoriales, neoplasmas de ventrículo cerebral tales como neoplasmas de plexo coloide, neoplasmas hipotalámicos y neoplasmas supratentoriales, neoplasmas meníngeos, neoplasmas de médula espinal los cuales incluyen neoplasmas epidurales, enfermedades demielinizantes tales como enfermedades de Canavan, esclerosis cerebral difusa la cual incluye adrenoleucodistrofia, encefalitis periaxialis, leucodistrofia de células globoides, esclerosis cerebral difusatal como leucodistrofia metacromática, encefalomielitis alérgica, encefalomielitis hemorrágica necrotizante, leucoencefalopatía multifocal progresiva, esclerosis múltiple, mielinolisis de pontina central, mielitis transversa, prurito lumbar, lordosis, síndrome de fatiga crónica, síndrome nervioso de presión alta, meningismo, enfermedades de la médula espinal tales como amiotonia congénita,

esclerosis lateral amiotrófica, atrofia muscular espinal tal como enfermedad de Werdnig-Hoffmann, compresión de médula espinal, neoplasmas de médula espinal tales como neoplasmas epidurales, siringomielia, tabes dorsal, síndrome del hombre rígido, retardo mental tal como síndrome de Angelman, síndrome de Cri-du-Chat, síndrome de De Lange, síndrome de Down, gangliosidosis tal como glangliosidosis G (M1), enfermedad de Sandhoff, enfermedad de Tay-Sachs, enfermedad de Hartup, homocistinuria, síndrome de Laurence-Moon Biedl, síndrome de Lesch-Nyhan, enfermedad urinaria del jarabe de arce, mucolipidosis tal como fucosidosis, lipofuscinocis ceroide neuronal, síndrome oculocerebrorenal, fenilcetonuria tal como fenilcetonuria maternal, síndrome de Prader-Willi, síndrome de Rett, síndrome de Rubistein-Taybi, esclerosis tuberosa, síndrome de WAGR, anormalidades del sistema nervioso tales como holoprosencefalia, defectos de tubos neurales tales como anencefalia la cual incluye hidrangencefalia, deformidad de arnold-Chairi, encefalocela, meningocele, meningomielocele, disrafismo espinal tal como espina bífida cistica y espina bífida oculta.

Enfermedades neurológicas adicionales que se pueden tratar o detectar con proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen neuropatías sensoriales y motoras hereditarias las cuales incluyen enfermedad de Charcot-Marie, atrofia óptica hereditaria, enfermedad de Refsum, paraplegia espástica hereditaria, enfermedad de Werdnig-Hoffmann, neuropatías autonómicas y sensoriales hereditarias tal como analgesia congénita y disautonomia familiar, manifestaciones neurológicas (tal como agnosia que incluye síndrome de Gerstmann, amnesia tal como amnesia retrograda, apraxia, vegiga neurogénica, cataplexia, trastornos comunicativos tales como trastornos de audición incluyendo sordera, pérdida auditiva parcial, loudness recruitment y tinitus, reclutamiento y tinnitus (tinteo), trastornos de lenguaje tales como afasia los cuales incluyen agrafia, anomia, afasia expresiva, y afasia de Wernicke, dislexia tal como dislexia adquirida, trastornos de desarrollo de lenguaje, trastornos del habla tal como afasia la cual incluye anomia, afasia expresiva y afasia de Wernicke, trastornos de articulación, trastornos comunicativos tales como trastornos del habla las cuales incluyen disartria, ecolalia, mutismo y tartamudeo, trastornos de voz tales como afonía y ronquera, estado descerebrado, delirio, fasciculación, alucinaciones, meningismo, trastornos del movimiento tal como síndrome de Angelman, ataxia, atetosis, corea, distonia, hipoquinesia, hipotonía muscular, mioclonía, tic, torticolis y temblor, hipertonia muscular tal como rigidez muscular tal como síndrome del hombre rígido, espasticidad muscular, parálisis tal como parálisis facial la cual incluye Herpes Zoster Otico, gastroparesia, hemiplegia, oftalmoplegia tal como diplopia, síndrome de Duane, síndrome de Horner, oftalmoplegia externa progresiva crónica tal como síndrome de Kearns, parálisis bulbar, paraparesia espástica tropical, paraplegia tal como síndrome de Brown-Sequard, cuadriplegia, paralisis respiratoria y paralisis de cuerdas vocales, paresia, extremidad fantasma, trastornos del gusto tales como agreusia y disgreusia, trastornos visuales tales como ambliopía, ceguera, defectos visuales de color, diplopia, hemianopsia, estocoma y visión subnormal, trastornos de sueño tales como hipersomnio el cual incluye Síndrome de Kleine-Leven, insomnio, sonambulismo, espasmo tal como trismo, inconciencia tal como coma, estado vegetal persistente, y síncople y vertigo, enfermedades neuromusculares tales como amiotonia congénita, esclerosis lateral amiotrófica, Sindrome Miasténico de Lambert-Eaton, enfermedad neuronal motora, atrofia muscular tal como atrofia muscular espinal, Enfermedad de Charcot-Marie y enfermedad de Werdning-Hoffmann, síndrome de postpoliomielitis, distrofia muscular, miastenia grave, miotonia atrófica, miotonia congenita, miopatía nemalina, paralisis periodica familiar, paramiloclono múltiple, paraparesia espástica tropical y síndrome del hombre rígido, enfermedades del sistema nervioso periférico tales como acrodinia, neuropatias amiloides, enfermedades del sistema nervioso autonómico tales

como síndrome de Adie, síndrome de Barre-Lieou, disautonomia familiar, síndrome de Horner, Distrofia Simpatética de Reflejo y Síndrome de Shy-Drager, Enfermedades de Nervios Craneales tales como Enfermedades de Nervios Acústicos tales como Neuroma Acústico el cual incluye Neurofibromatosis 2, Enfermedades de Nervios Faciales tales como Neuralgia Facial, Síndrome de Melkersson-Rosenthal, trastornos de motilidad ocular los cuales incluyen ambliopia, nistagmo, paralisis de nervios oculomotores, oftalmoplegia tal como síndrome de Duane, síndrome de Horner, Oftalmoplegia Externa Progresiva Crónica la cual incluye síndrome de Kearns, estrabismo tal como esotropia y exotropia, paralisis de nervios oculomotores, enfermedades nerviosas ópticas tal como atrofia óptica la cual incluye atrofia optica hereditaria, drusa de la papila óptica, neuritis óptica tal como neuromielitis óptica, papiledema, neuralgia trigeminal, parálisis de cuerdas vocales, enfermedades desmielizantes tal como neuromielitis óptica y lordosis, y neuropatías diabéticas tales como pie diabético.

Enfermedades neurológicas adicionales que se pueden tratar o detectar con proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albumina del invento incluyen síndromes de compresión nerviosa tal como síndrome de tuner carpiano, síndrome de salida toráxica tal como sindrome de costilla cervical, síndrome de compresión nerviosa ulnar, neuralgia tal como causalgia, neuralgia cervico-braquial, neuralgia facial y neuralgia trigeminal, neuritis tal como neuritis alérgica experimental, neuritis óptica, polineuritis, poliradiculoneuritis y radiculitis tal como poliradiculitis, neuropatias sensoriales y motoras hereditarias tal como enfermedad de Charcot-Marie, Atrofia Optica Hereditaria, Enfermedad de Refsum, Paraplegia Espástica Hereditaria y Enfermedad de Werdning-Hoffmann, Neuropatias Autonómicas y Sensoriales Hereditarias las cuales incluyen Analgesia Congenita y Disautonomia Familiar, Síndrome de POEMS, ciática, sudoración gustatoria y tétanos).

Trastornos Endocrinos

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se pueden utilizar para tratar, prevenir, diagnosticar, y/o pronosticar trastornos y/o enfermedades relacionadas con imbalance de hormonas, y/o trastornos o enfermedades del sistema endocrino.

Las hormonas secretadas por las glándulas del sistema endocrino controlan el crecimiento físico, función sexual, metabolismo, y otras funciones. Los trastornos se pueden clasificar en dos formas: perturbaciones en la producción de hormonas, y la incapacidad de tejidos para responder a hormonas. La etiología de estas enfermedades, trastornos o condiciones del sistema endocrino o imbalance de hormonas puede ser genética, somática, tal como cáncer y algunas enfermedades autoinmunes, adquirida (ej., mediante quimioterapia, lesión o toxinas), o infecciosa. Además, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar como un marcador o detector de un trastorno o enfermedad particular relacionada con el sistema endocrino y/o imbalance de hormonas.

El sistema endocrino y/o imbalance de hormonas y/o enfermedades contemplan trastornos de motilidad uterina incluyendo, pero sin limitar a: complicaciones con embarazo y parto (ej., parto prematuro, embarazo postérmino, aborto espontaneo, y parto lento o detenido); y trastornos y/o enfermedades del ciclo menstrual (ej., dismenorrea y endometriosis).

Las enfermedades y/o trastornos de imbalance de hormonas y/o sistema endocrino incluyen trastornos y/o enfermedades del pancreas, tales como, por ejemplo, diabetes mellitus, diabetes insipidus, agénesis pancreatica congenita, síndrome tumoral de células de isletas de feocromocitomas; trastornos y/o enfermedades de las glándulas adrenales tales como, por ejemplo, enfermedad de Addison, deficiencia corticosteroide, enfermedad virilizante, hirsutismo, síndrome de Cushing, hiperaldosteronismo, feocromocitoma, trastornos y/o enfermedades de la

glándula pituitaria, tal como, por ejemplo, hiperpituitarismo, hipopituitarismo, enanismo pituitario, adenoma pituitario, panhipopituitarismo, acromegalia, gigantismo; trastornos y/o enfermedades de la tiroides, incluyendo pero sin limitar a hipertiroidismo, hipotiroidismo, enfermedad de Plummer, enfermedad de Grave (bocio difuso tóxico), bocio nodular tóxico, tiroiditis (Tiroiditis de Hashimoto, tiroiditis granulomatosa subaguda, y tiroiditis linfocitica silenciosa), síndrome de Pendred, mixedema, cretinismo, tirotoxicosis, defecto de acoplamiento de hormona tiroides, aplasia tímica, tumores de células Hurthle de la tiroides, cáncer tiroideo, carcinoma tiroideo, cancinoma tiroideo medular; trastornos y/o enfermedades de la paratiroides, tal como, por ejemplo, hiperparatiroidismo, hipoparatiroidismo, trastornos y/o enfermedades del hipotalamo.

Además, los trastornos y/o enfermedades de imbalance de hormonas y/o sistema endocrino también pueden incluir trastornos y/o enfermedades de los testiculo u ovarios, incluyendo cáncer. Otros trastornos y/o enfermedades del los testiculos u avarios además incluyen, por ejemplo, cáncer ovárico, síndrome de ovario policístico, síndrome de Klinefelter, síndrome de testiculos evanescentes, (anorquidia bilateral), ausencia congenita de células de Leydig, criptorquidismo, síndrome de Nooman, distrofia miotónica, hemangioma capilar de los testiculos (benigno), neoplasias de los testiculos y neo-testículos.

Además, los trastornos y/o enfermedades del sistema endocrino y/o imbalance de hormonas también pueden incluir trastornos y/o enfermedades tales como, por ejemplo, síndromes de deficiencia poliglandular, feocromocitoma, neuroblastoma, neoplasia endocrina múltiple, y trastornos y/o cánceres de tejidos endocrinos.

En otra realización, las proteínas de fusión de albumina del invento y/o polinucleotidos que codifican proteinas de fusión de albúmina del invento, se pueden utilizar para diagnosticar, pronosticar, prevenir, y/o tratar enfermedades y/o trastornos endocrinos asociados con los tejidos en los cuales la proteina terapéutica que corresponde a la porción de proteína terapéutica de la proteína de albumina del invento es expresada.

Trastornos del sistema reproductivo

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para la diagnosis, tratamiento, o prevención de enfermedades y/o trastornos del sistema reproductivo. Los trastornos del sistema reproductivo que se pueden tratar mediante las composiciones del invento, incluyen, pero no están limitados a lesiones, infecciones, trastornos neoplásicos, defectos congenitos y enfermedades o trastornos del sistema reproductivo los cuales resultan en infertilidad, complicaciones con embarazo, trabajo de parto, parto, y dificultades después del parto.

Las enfermedades y/o trastornos del sistema reproductivo incluyen enfermedades y/o trastornos de los testiculos, incluyendo atrofia testicular, feminización testicular, criptorquidismo (unilateral y bilateral), anorquia, testiculos ectópicos, epididimitis y orquitis (tipicamente que resultan de infecciones tales como, por ejemplo, gonorrea, paperas, tuberculosis, y sifilis), torsión testicular, vasitis nodosa, tumores de células germinales (ej., semimonas, cancinomas celulares embrionarios, teratocarcinomas, coriocarcinomas, tumores del saco vitelino, y teratomas), turmores estromales (ej., tumores de células Leydig), hidrocele, hematocele, varicocele, espermatocele, hernia inguinal, y trastornos de producción de esperma (ej., síndrome de cilios inmóviles, aspermia, astenozoospermia, azoospermia, oligospermia, y teratozoospermia).

Trastornos del sistema reproductivo también incluyen trastornos de la glándula prostata, tal como prostatitis no bacteriana aguda, prostatitis no bacteriana crónica, prostatitis bacteriana aguada, prostatitis bacteriana crónica, prostatodistonia, prostatosis, prostatitis granulomatosa, malacoplaquia, hipertrofia prostática benigna o hiperplasia, y trastornos neoplásicos de la

prostata, incluyendo adenocarcinomas, carcinomas de células transicionales, carcinomas de ductos, y carcinomas de células escamosas.

Adicionalmente, las composiciones del invento pueden ser útiles en la dignosis, tratamiento, y/o prevención de trastornos o enfermedades del pene y uretra, incluyendo trastornos inflamatorios, tales como balanopostitis, blanitis xerótica obliterante, fimosis, parafimosis, sifilis, virus del herpes simplex, gonorrea, uretritis no gonococal, clamidia, micoplasma, VIH, SIDA, síndrome de Reitter, condiloma acumilatum, condiloma latum, papulas de pene nacaradas; anormalidades uretrales, tales como hipospadias, epispadias, y fimosis; lesiones premalignas, incluyendo eritroplasia de Queyrat, enfermedad de Bowen, paplosis bowenoide, condiloma gigante de Buscke-Lowenstein, y carcinoma varrucoso; cánceres del pene, incluyendo carcinomas celulares escamosos, carcinoma in situ, carcinoma verrucoso, y carcinoma de pene diseminado; trastornos neoplásicos uretrales, incluyendo carcinoma uretral de pene, carcinoma uretral bulbomembranoso, y carcinoma uretral prostático; y trastornos erectiles, tales como priapismo, enfermedad de Peyronie, disfunción erectil, e impotencia.

Además, las enfermedades y/o trastornos de los conductos deferentes incluyen vasculititis y CBAVD (ausencia bilateral congenita de conductos deferentes); adicionalmente, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar en la diagnosis, tratamiento, y/o prevención de enfermedades y/o trastornos de las vesiculas seminales, incluyendo enfermedad hidatídica, diarrea congenita de cloruro, y enfermedad renal policística.

Otros trastornos y/o enfermedades del sistema reproductor masculino incluyen, por ejemplo, síndrome de Klinefelter, síndrome de Young, eyaculación prematura, diabetes mellitus, fibrosis cistica, síndrome de Kartagener, fiebre alta, esclerosis múltiple y ginecomastia.

Además, los polinucleotidos, proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar en la diagnosis, tratamiento, y/o prevención de enfermedades y/o trastornos de la vagina y vulva, incluyendo vaginosis bacteriana, vaginitis candida, virus del herpes simplex, chancroide, granuloma inguinal, linfogranuloma venereum, sarna, papilomavirus humano, trauma vaginal, trauma vulvar, adenosis, viginitis, clamidia, gonorrea, vaginitis tricomona, condiloma acuminatum, sífilis, molusco contagioso, vaginitis atrófica, enfermedad de Paget, esclerosis líquen, líquen planus, vulvodinia, síndrome de choque tóxico, viginismo, vulvovaginitis, vestibulitis vulvar, y trastornos neoplásicos, tales como hiperplasia celular escamosa, carcinoma celular claro, carcinoma celular basal, melanomas, cáncer de la glándula de Bartholin, y neoplasia intraepitelial vulvar.

Trastornos y/o enfermedades del utero incluyen dismenorrea, útero retrovertido, endometriosis, fibroides, adenomiosis, sangrado anovulatorio, amenorrea, síndrome de Cushing, mole hidatidiforme, síndrome de Asherman, menopausia prematura, pubertad precoz, pólipos uterinos, sangrado uterino disfuncional (ej., debido a señales hormonales aberrantes), trastornos neoplásicos, tales como adenocarcinomas, queimiosarcomas, y sarcomas. Adicionalmente, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden ser útiles como un marcador o detector de, así como también en la diagnosis, tratamiento, y/o prevención de anormalidades uterinas congenitas, tal como utero bicornudo, utero septado, utero unicornado simple, utero unicornado con un cuerno rudimentario sin comunicación entre cavidades, útero unicornado con un cuerno con comunicación entre cavidades, útero unicornado con un cuerno con comunicación entre cavidades, útero arqueado, didelfo uterino, y útero en forma de T.

Trastornos y enfermedades ováricas incluyen anovulación, síndrome de ovario policístico (síndrome de Stein-Leventhan), quistes ováricos, hipofunción ovárica, insensibilidad

ovárica a gonadotropinas, sobreproducción ovárica de andrógenos, síndrome de vena ovárica derecha, amenorrea, hirsutismo y cáncer ovárico (incluyendo, pero sin limitar a crecimiento canceroso primario y secundario, tumores Sertoli-Leydig, carcinoma endometroide del ovario, adenocarcinoma seroso papilar ovárico, adenocarcinoma mucinoso ovárico, y tumores ováricos de Krukenberg).

Enfermedades y/o trastornos cervicales incluyen cervisitis, cervisitis crónica, cervisitis mucopurulenta, displasia cervical, pólipos cervicales, quistes Nabotianos, erosión cervical, incompetencia cervical, y neoplasmas cervicales (incluyendo, por ejemplo, carcinoma cervical, metaplasia escamosa, carcinoma de células escamosas, neoplasia de células adenoescamosas, y neoplasia celular columnar).

Adicionalmente, los trastornos y/o enfermedades del sistema reproductivo incluyen trastornos y/o enfermedades del embarazo, incluyendo aborto y mortinato, tal como aborto temprano, aborto tardío, aborto espontáneo, aborto inducido, aborto terapéutico, amenaza de aborto, aborto fallido, aborto incompleto, aborto habitual y aborto séptico; embarazo ectópico, anemia, incompatibilidad de Rh, sangrado vaginal durante embarazo, diabetes gestacional, retardo de crecimiento intrauterino, polihidramnios, síndrome HELLP, ablatio placentae, placenta previa, hiperemesia, preeclampsia, eclampsia, herpes gestationis, y urticaria de embarazo. Adicionalmente, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar en la diagnosis, tratamiento, y/o prevención de enfermedades que pueden complicar el embarazo, incluyendo enfermedades cardiacas, falla cardiaca, enfermedad cardiaca reumática, enfermedad cardiaca congénita, prolapso de válvula mitral, presión sanguínea alta, enfermedad renal, enfermedad infecciosa (ej., rubéola, citomegalovirus, toxoplasmosis, hepatitis infecciosa, clamidia, VIH, SIDA, y herpes genital), diabetes mellitus, enfermedad de Grave, tiroiditis, hipotiroidismo, tiroiditis de Hashimoto, hepatitis activa crónica, cirrosis hepática, cirrosis biliar primaria, asma, lupus sistémico eritematoso, artritis reumatoide, miastenia grave, purpura trombocitopénica yodopática, apendicitis, quistes ováricos, trastornos de la vesícula biliar, y obstrucción del intestino.

Complicaciones asociadas con el trabajo de parto y parto incluyen ruptura prematura de las membranas, parto prematuro, embarazo postérmino, postmadurez, parto que avanza muy lento, distrés fetal (ej., tasa cardiaca anormal (fetal o maternal), problemas respiratorios, y posición fetal anormal), distocia de hombro, cordón umbilical prolapsado, embolismo de fluido amniótico, y sangrado uterino aberrante.

Además, enfermedades y/o trastornos del período postparto, incluyen endometritis, parametritis, peritonitis, tromboflebitis pélvica, embolismo pulmonar, endotoxemia, pielonefritis, tromboflebitis safenosa, mastitis, cistitis, hemorragia postparto, y útero invertido.

Otros trastornos y/o enfermedades del sistema reproductivo femenino que se pueden diagnosticar, tratar, y/o prevenir mediante las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen, por ejemplo, síndrome de Turner, pseudohermafrodismo, síndrome premenstrual, enfermedad inflamatoria pélvica, congestión pélvica (congestión vascular), frigidez, anorgasmia, dispaneuria, trompa de falopio rota, y Mittelschmerz.

Enfermedad Infecciosa

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para tratar o detectar agentes infecciosos. Por ejemplo, aumentando la respuesta inmune, particularmente aumentando la

proliferación y diferenciación de células B y/o T, se pueden tratar de las enfermedades infecciosas. La respuesta inmune se puede aumentar ya sea al aumentar una respuesta inmune existente, o al aumentar una respuesta inmune nueva. Alternativamente, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento también pueden inhibir directamente el agente infeccioso, sin producir necesariamente una respuesta inmune.

Los virus son un ejemplo de un agente infeccioso que pueden producir enfermedad o síntomas que se pueden tratar o detectar mediante proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento. Ejemplos de virus, incluyen, pero no están limitados a virus de ADN o ARN y familias virales: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, VIH, Flaviridae, Hepadnaviridae (Hepatitis), Herpesviridae (tal como, Citomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (ej., Paramixoviridae, Morbillivirus, Rhabdoviridae), Ortomixoviridae (ej., Influenza A, Influenza B, y parainfluenza), virus Papiloma, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae), (tal como Viruela o Vaccinia), Reoviridae (ej., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), Togaviridae (ej., Rubivirus). Los virus que están en estas familias pueden producir una variedad de enfermedades o síntomas, incluyendo, pero sin limitar a: artritis, bronquiolitis, virus cincitial respiratorio, encefalitis, infecciones oculares (ej., conjuntivitis, queratitis), síndrome de fatiga crónica, hepatitis (A, B, C, E, Activa Crónica, Delta), encefalitis japonesa B, Junin, Chikungunya, fibre del Valle Rift, fiebre amarilla, miringitis, infecciones oportunistas (ej., SIDA), neumonía, linfoma de Burkitt, viruela de pollo, fiebre hemorragica, sarampión, paperas, parainfluenza, hidrofobias, resfriado común, polio, leucemia, rubéola, enfermedades de transmisión sexual, enfermedades dérmicas (ej., verrugas de Kaposi), y viremia. Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se pueden utilizar para tratar o detectar cualquiera de estos síntomas o enfermedades. En realizaciones específicas, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan para tratar: meningitis, dengue, EBV, y/o hepatitis (ej., hepatitis B). En una realización específica adicional las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan para tratar pacientes que no responden a una o más vacunas de hepatitis comercialmente disponibles. En una realización específica adicional las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan para tratar SIDA.

De manera similar, agentes bacterianos y micóticas que pueden producir enfermedades o síntomas y que se pueden tratar o detectar mediante proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen, pero no están limitados a bacterias gram-Negativas y Gram-positivas, familias bacterianas, y hongos: Actinomyces (ej., Norcardia), Acinetobacter, *Criptococcus neoformans,* Aspergillus, Bacillaceae (ej., *Bacillus anthrasis), Bacteriodes fragilis*), Blastomicosis, Bordetella, Borrelia (ej., *Borrelia burgdorferi)*, Brucella, Candidia, Campylobacter, Clamidia, Clostridio (ej., *Clostridium botulinum, Clostridium dificile, Clostridium perfringens, Clostridium tetani*), Coccidioides, Corinebacterium (ej., *Corynebacterium diphtheriae*), Dermatocicosis, *E. coli* (ej., *E. coli* enterotoxigénica y *E. coli* Enterohemorrágica), Enterobacter (ej., *Enterobacter aerogenes),* Enterobacteriaceae (Klebsiella, Salmonella (ej., *Salmonella typhi, Salmonella enteritidis, Salmonella typhy*), Serratia, Yersinia, Shigella), Erysipelothrix, Haemophilus (ej., *Haemophilus influenza* tipo B), Helicobacter, Legionella (ej., *Legionella pneumophila*), Leptospira, Listeria

(ej., *Listeria monocitogenes*), Micoplasma, Micobacterium (ej., *Mycobacterium leprae* y *Mycobacterium tuberculosis*), Vibrio (ej., *Vibrio cholerae*), Neisseriaceae (ej., *Neisseria gonorrhea, Neisseria meningitidis*), Pasteurellacea, Proteus, Pseudomonas (ej., *Pseudomonas aeruginosa*), Rickettsiaceae, Spirochetes (ej., Treponema spp., Leptospira spp., Borrelia spp.), Shigella spp., Staphyococcus (ej., *Stafpylococcus aureus*), Meniniococcus, Pneumococcus y Streptococcus (ej., *Streptococcus pneumoniae* y Streptococcus Grupos A, B, y C), y Ureaplasmas. Estas familias bacterianas, parasíticas, y micóticas pueden producir enfermedades o síntomas, incluyendo, pero sin limitar a: infecciones resistentes a antibióticos, bacteremia, endocarditis, septicemia, infecciones oculares (ej., conjuntivitis), uveitis, tuberculosis, gingivitis, diarrea bacteriana, infecciones oportunistas (ej., infecciones relacionadas con SIDA), paroniquia, infecciones relacionadas con prótesis, caries dental, enfermedad de Reiter, infecciones del tracto respiratorio, tal como Whooping Cough o Empiema, sepsis, enfermedad de Lyme, enfermedad de rasguño de gato, disentería, fiebre paratifoidea, envenenamiento por alimentos, enfermedad de Legionella, inflamación crónica y aguda, eritema, infecciones de levadura, tifoideo, neumonía, gonorrea, meningitis (ej., miringitis tipos A y B), clamidia, sífilis, difteria, lepra, brucelosis, úlceras pépticas, antrax, abortos espontáneos, defectos de nacimiento, neumonía, infecciones pulmonares, infecciones de oídos, ceguera, letargo, malestar, vómito, diarrea crónica, enfermedad de Crohn, colitis, vaginosis, esterilidad, enfermedades inflamatorias pélvicas, candidiasis, paratuberculosis, tuberculosis, lupus, botulismo, gangrena, tétanos, impétigo, fiebre reumática, escarlatina, enfermedades de transmisión sexual, enfermedades dermatológicas (ej., celulitis, dermatocicosis), toxemia, infecciones del tracto urinario, infecciones de heridas, infecciones nosocómicas. Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se pueden utilizar para tratar o detectar cualquiera de estos síntomas o enfermedades. En realizaciones específicas, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para tratar: tétanos, difteria, botulismo, y/o meningitis tipo B.

Además, los agentes parásitos que producen enfermedades o síntomas que se pueden tratar, prevenir, y/o diagnosticar mediante proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento incluyen, pero no están limitados a, las siguientes familias o clases: Amebiasis, Babesiosis, Coccidiosis, Criptosporidiosis, Dientamoebiasis, sífilis equina, ectoparasitica, giardias, helmintiasis, leishmaniasis, esquistisoma, fiebre de la costa oriental, toxoplasmosis, tripanosomiasis y tricomonas y sporozoans (ej., *plasmodium virax, plasmodium faciparium, plasmodium malariae* y *plasmaodium ovale).* Estos parásitos pueden causar una variedad de enfermedades o síntomas, incluyendo, pero sin limitar a: sarna, trombiculiasis, infecciones oculares, enfermedad intestinal (ej., disentería, giardiasis, enfermedad hepática, enfermedad pulmonar, infecciones oportunistas (ej., relacionadas con SIDA), malaria, complicaciones del embarazo, y toxoplasmosis. Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se pueden utilizar para tratar, prevenir, y/o diagnosticar cualquiera de estos síntomas o enfermedades. En realizaciones específicas, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se utilizan para tratar, prevenir, y/o diagnosticar malaria.

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden administrar en una cantidad efectiva al paciente, o al remover células del paciente, proporcionando las células con un polinucleotido del presente invento, y retornando las células modificadas al paciente (terapia *ex vivo*). Además, las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión

de albúmina del invento se pueden utilizar como un antígeno en una vacuna para elevar una respuesta inmune contra una enfermedad infecciosa.

Regeneración

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar para diferenciar, proliferar, y atraer células, llevando a la regeneración de tejidos. (Ver, Science 276:59-87 (1997)). La regeneración de tejidos se puede utilizar para reparar, reemplazar, o proteger tejido dañado por defectos congenitos, trauma (heridas, quemaduras, incisiones, o úlceras), edad, enfermedad (ej., osteoporosis, osteocartritis, enfermedad periodontal, falla hepática), cirugia, incluyendo cirugia plástica cosmética, fibrosis, lesión de reperfusión, o daño sistémico de citoquinas.

Los tejidos que se pueden regenerar utilizando el presente invento incluyen tejido de órganos (ej., páncreas, hígado, intestino, riñón, piel, endotelio), muscular (suave, óseo, o cardiaco), vascular (incluyendo vascular y linfático), nervioso, hematopoyetico, y óseo (hueso, cartilago, tendón, y ligamento). Preferiblemente, la regeneración ocurre sin cicatrización o cicatrización disminuida. La regeneración también puede incluir angiogénesis.

Además, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, pueden aumentar la regeneración de tejidos difíciles de curar. Por ejemplo, la regeneración aumentada de tendones/ligamentos aceleraría el tiempo de recuperación después de daño. Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento también se podrían utilizar de manera profiláctica en un esfuerzo por evitar daño. Enfermedades específicas que se podrían tratar incluyen tendinitis, síndrome del túnel del Carpio, y otros defectos de tendones o ligamentos. Un ejemplo adicional de regeneración de tejidos de heridas que no curan incluye úlceras de presión, úlceras asociadas con insuficiencia vascular, heridas quirúrgicas y traumáticas.

De manera similar, el tejido nervioso y cerebral también se puede regenerar utilizando proteínas de fusión del invento y/o polinucleotidos que codifican protreínas de fusión de albúmina del invento, para proliferar y diferenciar células nerviosas. Las enfermedades que se pueden tratar utilizando este método incluyen enfermedades del sistema nervioso central y periférico, neuropatías, o trastornos mecánicos y traumáticos (ej., trastornos de médula espinal, trauma de cabeza, enfermedad cerebrovascular, y apoplejía). Específicamente, enfermedades asociadas con lesiones nerviosas periféricas, neuropatía periférica (ej., que resulta de quimioterapia u otras terapias médicas), neuroterapias localizadas, y enfermedades del sistema nervioso central (ej., enfermedad de Alzheimer, enfermedad de Parkinson, enfermedad de Huntington, esclerosis lateral amiotrófica, y síndrome de Shy-Grager), podrían todas ser tratadas utilizando proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento.

Trastornos Gastrointestinales

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se pueden utilizar para tratar, prevenir, diagnosticar, y/o pronosticar trastornos gastrointestinales, incluyendo enfermedades y/o condiciones inflamatorias, infecciones, cánceres (ej., neoplasmas intestinales (tumor carcinoide del intestino delgado, linfoma no Hodgking del intestino delgado, linfoma de tasa pequeña)), y úlceras, tales como úlceras pépticas.

Los trastornos gastrointestinales incluyen disfagia, odinofagia, inflamación del esófago, esofagitis péptica, reflujo gástrico, fibrosis submucosal y estrictura. lesiones Mallory-Weiss, leiomiomas, lipomas, cánceres epidérmicos, adenocarcinomas, trastornos de retención gástrica, gastroenteritis, atrofia gástrica, cánceres gástricos estomacales, pólipos del estómago, trastornos

autoinmunes tales como anemia perniciosa, estenosis pilórica, gastritis (bacteriana, viral, eosinofílica, inducida por estrés, erosiva crónica, atrófica, de células de plasma, y de Ménétrier), y enfermedades peritoneales (ej., quiloperoneo, hemoperitoneo, quiste mesentérico, linfadenitis mesentérica, oclusión vascular mesentérica, paniculitis, neoplasmas, peritonitis, pneumoperitoneo, absceso bubfrénico).

Trastornos gastrointestinales también incluyen trastornos asociados con el intestino delgado, tal como síndrome de mala absorción, distensión, síndrome de intestino irritable, intolerancia al azúcar, enfermedad celiaca, úlceras duodenales, duodenitis, psilosis tropical, enfermedad de Whipple, linfangiectasia intestinal, enfermedad de Crohn, apendicitis, obstrucciones del ileo, divertículum de Meckel, divertícula múltiple, falla de rotación completa del intestino delgado y grueso, linfoma, enfermedades bacterianas y parásitas (tal como diarrea de Traveler, tifoideo y paratifoideo, cólera, infección de gusanos redondos (*Ascariasis lumbricoides*), anquilostomas (*enterobius vermicularis*), gusanos planos (*Taenia saginata, Equinococcus granulosus, Diphyllobothrium spp.*, y *T. solium*).

Enfermedades y/o trastornos hepáticos incluyen colestasis intrahepática (síndrome de alagilla, cirrosis hepática biliar), hígado graso (hígado graso alcóholico, síndrome de Reye), trombosis de vena hepática, degeneración hepatolentricular, hepatomegalia, síndrome hepatopulmonar, síndrome hepatorenal, hipertensión portal (varices esofageas y gástricas), absceso hepático (absceso hepático amebico), cirrosis hepática (alcóholica, biliar y experimental), enfermedades hepáticas alcohólicas (hígado graso, hepatitis, cirrosis), parásitas (equinococosis hepática, fascioliasis, absceso hepático amébico), ictericia (hemolítica, hepatocelular, y colestática), colestasis, hipertensión portal, agrandamiento hepático, ascites, hepatitis (hepatitis alcohólica, hepatitis animal, hepatitis crónica (autoinmune, hepatitis B, hepatitis C, hepatitis D, inducida por fármacos), hepatitis tóxica, hepatitis humana viral (hepatitis A, hepatitis B, hepatitis C, hepatitis D, hepatitis E), enfermedad de Wilson, hepatitis granulomatosa, cirrosis biliar secundaria, encefalopatía hepática, hipertensión portal, varices, encefalopatía hepática, cirrosis biliar primaria, colangitis esclerosante primaria, adenoma hepatocelular, hemangiomas, cálculos biliares, falla hepática (encefalopatía hepática, falla hepática aguda), y neoplasmas hepáticos (angiomiolipoma, metástasis hepáticas calcificadas, metástasis hepáticas sísmicas, tumores epiteliales, hepatocarcinoma fibrolaminar, hiperplasia nodular focal, adenoma hepático, cistadenoma hepatobiliar, hepatoblastoma, carcinoma hepatocelular, hepatoma, cáncer hepático, hemangioendotelioma hepático, hamartoma mesenquimal, tumores mesenquimales del hígado, hiperplasia regenerativa nodular, tumores hepáticos benignos (quistes hepáticos [quistes simples, enfermedad hepática policística, cistadenoma hepatobiliar, quiste coledocal], tumores mesenquimales [hamartoma mesenquimal, hemangioendotelioma infantil, hemangioma, Peliosis Hepatis, Lipomas, pseudomotor Inflamatorio, Miscelaneo], tumores epiteliales [epitelio del ducto biliar (hamartoma de ducto biliar, adenoma de ducto biliar), Hepatocito (Adenoma, hiperplasia nodular focal, hiperplasia regenerativa nodular)], tumores hepáticos malignos [hepatocelular, hepatoblastoma, carcinoma hepatocelular, colangiocelular, colangiocarcinoma, cistadenocarcinoma, tumores de vasos sanguineos, angiosarcoma, sarcoma de Kaposi, hemangioendotelioma, otros tumores, sarcoma embrional, fibrosarcoma, leiomiosarcoma, rabdomiosarcoma, carcinosarcoma, teratoma, carcinoide, carcinoma escamoso, linfoma primario]), peliosis hepatis, porfiria eritrohepática, porfiria hepática (porfiria intermitente aguda, porfiria cutánea tarda), síndrome de Zellweger).

Trastornos y/o enfermedades pancreáticas incluyen pancreatitis aguda, pancreatitis crónica (pancreatitis necrotizante aguda, pancreatitis alcohólica), neoplasmas (adenocarcinoma del páncreas, cistadenocarcinoma, insulinoma, gastrinoma, y glucagonoma, neoplasmas císticos,

tumores de células de isletas, pancreoblastoma), y otras enfermedades pancreáticas (ej., fibrosis cistica, quiste (pseudoquiste pancreatico, fístula pancreática, insuficiencia)).

Enfermedades de la vesícula biliar incluyen cálculos (colelitiasis y coledocolitiasis), síndrome de postcolecistectomia, diverticulosis de la vesícula biliar, colecistitis aguda, colecistitis crónica, tumores del ducto biliar, y mucocele.

Enfermedades y/o trastornos del intestino grueso incluyen colitis asociada con antibióticos, diverticulitis, colitis ulcerativa, megacolon adquirido, abscesos, infecciones micóticas y bacterianas, trastornos anorectales, (ej., fisuras, hemorroides), enfermedades colónicas (colitis, neoplasmas colónicos [cáncer de colon, pólipos adenomatosos de colon (ej., adenoma velloso), carcinoma de colon, cáncer colorectal], diverticulitis colónica, diverticulosis colónica, megacolon [enfermedad de Hirschsprung, megacolon tóxico]; enfermedades sigmoides [proctocolitis, neoplasmas de sigmoin]), constipación, enfermedad de Crohn, diarrea (diarrea infantil, disentería), enfermedades duodenales (neoplasmas duodenales, obstrucción duodenal, úlcera duodenal, duodenitis), enteritis (enterocolitis), enteropatía de VIH, enfermedades del íleon (neoplasmas del íleon, ileitis), enfermedad del intestino delgado inmunoproliferativa, enfermedad intestinal inflamatoria (colitis ulcerativa, enfermedad de Crohn), atresia intestinal, enfermedades parásitas (anisaquiasis, balantidiasis, blastocistis, criptosporidiosis, dientamoebiasis, disentería amébica, giardiasis), fístula intestinal (fístula rectal), neoplasmas intestinales (neoplasmas cecales, neoplasmas colónicos, neoplasmas duodenales, neoplasmas íleos, pólipos intestinales, neoplasmas yeyunales, neoplasmas rectales, obstrucción intestinal (síndrome de vuelta aferente, obstrucción duodenal, heces impactadas, pseudo-obstrucción intestinal [cecal volvulus], indigitación), perforación intestinal, pólipos intestinales (pólipos colónicos, síndrome de gardner, síndrome de peutz-jeghers), síndromes de mala absorción (síndrome de bucle ciego, enfermedad celiaca, intolerancia a lactosa, síndrome de la tasa corta, psilosis tropical, enfermedad de Whipple), oclusión vascular mesentérica, pneumatosis cistoides intestinalis, protein-losing enteropathies de pérdidas de proteínas (linfangiectasis intestinal), enfermedades rectales (enfermeades del ano, incontinencia fecal, hemorroides, proctitis, fístula rectal, prolapso rectal, rectocele), úlcera péptica (úlcera duodenal, esofagitis péptica, hemorragia, perforación, úlcera estomacal, síndrome de Zollinger-Ellison), síndromes de postgastrectomia (síndrome de descarga), enfermedades estomacales (ej., aclorhidria, reflujo duodenogástrico (reflujo biliar), ectasia vascular antral gástrica, fístula gastrica, obstrucción de salida gástrica, gastritis (atrófica o hipertrófica), gastroparesia, dilatación estomacal, divertículo estomacal, neoplasmas estomacales (cáncer gástrico, pólipos gástricos, adenocarcinoma gástrico, pólipo gástrico hiperplásico), ruptura estomacal, úlcera estomacal, vólvulo estomacal), tuberculosis, visceroptosis, vómito (ej., hematemesis, hiperémesis gravidarum, nausea y vómito postoperativo) y colitis hemorrágica.

Trastornos y/o enfermedades adicionales del sistema gastrointestinal incluyen enfermedades del tracto biliar, tales como, gastrosquisis, fístula (ej., fístula biliar, fístula esofágica, fístula gástrica, fístula intestinal, fístula pancreática), neoplasmas (ej., neoplasmas del tracto biliar, neoplasmas esofágicos, tales como adenocarcinoma del esófago, carcinoma celular escamoso esofágico, neoplasmas gastrointestinales, neoplasmas pancreáticos, tales como adenocarcinoma del páncreas, neoplasma cístico mucinoso del páncreas, neoplasmas císticos pancreáticos, pancreatoblastoma, y neoplasmas peritoneales), enfermedad esofágica (ej., enfermedades bulosas, candidiasis, acantosis glicogénica, ulceración, varices de esofago de barret, atresia, quistes, divertículo (ej., divertículo de Zenker), fístula (ej., fístula traqueoesofágica), trastornos de motilidad (ej., síndrome de CREST, trastornos de deglución, acalasia, espasmo, reflujo gastroesofágico), neoplasmas, perforación (ej., síndrome de Boerhaave,

síndrome de Mallory-Weis), estenosis, esofagitis, hernia diafragmática (ej., hernia hiatal); enfermedades gastrointestinales, tales como, gastroenteritis (ej., cólera morbo, infección de virus de Norwalk), hemorragia (ej., hematemesis, melena, hemorragia de úlcera péptica), neoplasmas estomacales (cáncer gástrico, pólipos gástricos, adenocarcinoma gástrico, cáncer estomacal)), hernia (ej., hernia diafragmática congénita, hernia femoral, hernia inguinal, hernia obturadora, hernia umbilical, hernia ventral), y enfermedades intestinales (ej., enfermedades cecales (apendicitis, neoplasmas cecales)).

Quimiotaxis

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden tener actividad quimiotáxica. Una molécula quimiotáxica atrae o moviliza células (ej., monocitos, fibroblastos, neurotrofilos, células-T, células mast, eosinofilos, células epiteliales y/o endoteliales) a un sitio particular en el cuerpo, tal como inflamación, infección, o sitio de hiperproliferación. Luego las células movilizadas pueden repeler y/o curar la anormalidad o trauma particular.

Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden aumentar la actividad quimiotáxica de células particulares. Luego estas moléculas quimiotáxicas se pueden utilizar para tratar inflamación, infección, trastornos hiperproliferativos, o cualquier trastorno del sistema inmune al aumentar el número de células dirigidas a una locación particular en el cuerpo. Por ejemplo, las moléculas quimiotáxicas se pueden utilizar para tratar heridas y otros traumas de tejidos mediante la atracción de células inmunes hacia la locación lesionada. Las moléculas quimiotácticas del presente invento también pueden atraer fibroblastos, los cuales se pueden utilizar para tratar heridas.

También se contempla que las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento pueden inhibir actividad quimiotáctica. Estas moléculas también se podrían utilizar para tratar trastornos. Por lo tanto, las proteínas de fusión del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento se pueden utilizar como un inhibidor de quimiotaxis.

Actividad de Unión

Las proteínas de fusión de albúmina del invento se pueden utilizar para seleccionar moléculas que se unen a la porción de proteínas terapéuticas de la proteína de fusión o moleculas a las cuales la porción de proteínas terapéuticas de la proteína de fusión se une. La unión de la proteína de fusión y la molécula puede activar (agonista), aumentar, inhibir (antagonista), o disminuir la actividad de la proteína de fusión o la molécula unida. Ejemplos de tales moléculas incluyen anticuerpos, oligonucleotidos, proteínas (ej., receptores), o moleculas pequeñas.

Preferiblemente, la molécula es estrechamente relacionada con el ligando natural de la porción de proteína terapéutica de la proteína de fusión del invento, ej., un fragmento del ligando, o un sustrato natural, un ligando, un mimético estructural o funcional. (Ver, Coligan, Protocolos Actuales en Inmunologia 1(2): Capitulo 5 (1991)). De manera similar, la molécula puede estar estrechamente relacionada con el receptor natural al cual la porción de proteína terapéutica de una proteína de fusión de albúmina del invento se une, o al menos, un fragmento del receptor capaz de ser unido por la porción de proteína terapéutica de una proteína de fusión de albúmina del invento (ej., sitio activo). En cualquier caso, la molécula puede ser diseñada de manera racional utilizando técnicas conocidas.

Preferiblemente, la selección de estas moléculas involucra producción de células apropiadas que expresen las proteínas de fusión de albúmina del invento. Las células preferidas incluyen células de mamíferos, levadura, Drosophila, o *E. coli*.

El ensayo simplemente puede probar la unión de un compuesto candidato a una proteína de fusión de albúmina del invento, en donde la unión es detectada por un rótulo, o en un ensayo que involucra competencia con un competidor rotulado. Además, el ensayo puede probar si el compuesto candidato resulta en una señal generada mediante unión a la proteína de fusión.

Alternativamente, el ensayo se puede realizar utilizando preparaciones libres de células, proteinas de fusión/moleculas fijadas a un soporte sólido, bibliotecas químicas, o mezclas de productos naturales. El ensayo simplemente puede comprender los pasos de mezclar un compuesto candidato con una solución que contiene una proteína de fusión de albumina, midiendo actividad o unión de proteina/molecula, y comparando la actividad o unión de proteina de fusión/molecula con un estándar.

Preferiblemente, un ensayo ELISA puede medir la actividad o nivel de proteina de fusión en una muestra (ej., muestra biológica) utilizando un anticuerpo monoclonal o policlonal. En anticuerpo puede medir el nivel o actividad de proteína de fusión ya sea al unir, directa o indirectamente a la proteína de fusión de albúmina o al competir con la proteína de fusión de albúmina por un sustrato.

Adicionalmente, el receptor al cual una porción de proteína terapéutica de una proteína de fusión de albúmina del invento se une puede ser identificado mediante numerosos métodos conocidos por aquellos con experiencia en el estado de la técnica, por ejemplo, toma panorámica de ligandos y clasificación FACS (Coligan, Current Protocols in Immun., 1(2), Capitulo 5, (1991)). Por ejemplo, en casos donde la porción de proteína terapéutica de la proteína fusión corresponde a FGF, se puede emplear clonación de expresión en donde el ARN poliadenilado es preparado a partir una célula sensible a la proteina de fusión de albúmina, por ejemplo, las células NIH3T3 las cuales se sabe contienen múltiples receptores para las proteinas de la familia FGF, y células SC-3, y una biblioteca de cADN creada a partir de este ARN es dividida en grupos y utilizada para transfectar células COS u otras células que no son sensibles a la proteína de fusión de albumina. Las células transfectadas que son cosechadas sobre portaobjetos de vidrio son expuestas a la proteina de fusión de albumina del presente invento, después de ser rotuladas. Las proteínas de fusión de albúmina se pueden rotular mediante una variedad de medios incluyendo tratamiento con yodo o inclusión de un sitio de reconocimiento para una proteinquinasa específica a sitio.

Después de fijación e incubación, los portaobjetos son sometidos a análisis auto-radiográfico. Se identifican grupos positivos y sub-grupos son preparados y re-transfectados utilizando un proceso iterativo de sub-agrupamiento y re-clasificación, eventualmente produciendo un clon simple que codifica el receptor putativo.

Como un enfoque alternativo para identificación de receptor, una proteína de fusión de albúmina rotulada puede ser ligada por fotoafinidad con membrana celular o preparaciones de extractos que expresen la molecula receptora para el componente de proteina Terapéutica de una proteína de fusión de albúmina del invento, el material ligado puede ser determinado mediante análisis PAGE y expuesto a pelicula de rayos-X. El complejo rotulado que contiene los receptores de la proteína de fusión puede ser extirpado, resuelto en fragmentos peptidos, y sometido a microsecuenciamiento proteico. La secuencia de aminoácidos obtenida a partir de microsecuenciamiento sería utilizada para diseñar un grupo de sondas oligonucleotidas degradadas para clasificar una biblioteca de cADN para identificar los genes que codifican los receptores putativos.

Además, las técnicas de mezclado de genes, mezclado de motivos, mezclado de exonas, y/o mezclado de codones (colectivamente denominado "mezclado de ADN") se pueden emplear para modular las actividades de proteinas de fusión, y/o porción de proteína Terapéutica o

componente de albúmina de una proteína de fusión de albúmina del presente invento, generando de manera efectiva agonistas y antagonistas de una proteína de fusión de albúmina del presente invento. *Ver generalmente,* Patentes Americanas No.5.605.793, 5.811.238, 5.830.721, 5.834.252, y 5.837.458, y Patten, P. A., *et al., Curr. Opinion Biotechnol.* 8:724-33 (1997); Harayama, S. *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, L. O., *et al.,* J. Mol. Biol. 287:265-76 (1999); y Lorenzo, M. M. y Blasco, R. *Biotechniques* 24(2):308-13 (1998); cada una de estas patentes y publicaciones son incorporadas en la presente como referencia). En una realización, la alteración de polinucleotidos que codifican proteínas de fusión de albúmina del invento y por lo tanto, las proteinas de fusión de albúmina codificadas así, se puede lograr mediante mezclado de ADN. El mezclado de ADN involucra el ensamble de dos o más segmentos de ADN en una molecula deseada mediante recombinación homologa, o específica a sitio. En otra realización, los polinucleotidos que codifican proteinas de fusión de albúmina del invento y por lo tanto, las proteínas de fusión de albúmina codificadas así, se pueden alterar al ser sometidas a mutagenesis aleatoria mediante PCR propensa a error, insercción nucleotida aleatoria u otros métodos antes de recombinación. En otra realización, uno o más componentes, motivos, secciones, partes, dominios, fragmentos, etc., de una proteina de fusión de albúmina del presente invento se pueden recombinar con uno o más componentes, motivos, secciones, partes, dominios, fragmentos, etc. de una o más moleculas heterólogas. En realizaciones preferidas, las moleculas heterólogas son miembros de la familia. En realizaciones preferidas adicionales, la molécula heteróloga es un factor de crecimiento tal como, por ejemplo, factor de crecimiento derivado de plaquetas (PDGF), factor de crecimiento tipo insulina (IGF-I), factor de crecimiento de transformación (TGF)-alfa, factor de crecimiento epidérmico (EGF), factor de crecimiento de fibroblastos (FGF), TGF-beta, proteína morfogenética ósea (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A y B, decapentaplegico (dpp), 60A, OP-2, dorsalina, factores de diferenciación de crecimiento (GDFs), nodal, MIS, TGF-beta, TGF-beta2, TGF-beta3, TGF-beta5, y factor neurotrófico glial-derivado (GDNF).

Otros fragmentos preferidos son fragmentos biologicamente activos de la porción de proteína Terapéutica y/o componente de albúmina de las proteínas de fusión de albúmina del presente invento. Los fragmentos biologicamente activos son aquellos que exhiben actividad similar, pero no necesariamente identica, a una actividad de una porción de proteína Terapeutica y/o componente de albúmina de las proteínas de fusión de albumina del presente invento. La actividad biológica de los fragmentos puede incluir una actividad deseada mejorada, o una actividad indeseada disminuida.

Adicionalmente, este invento proporciona un método de clasificación de compuestos para identificar aquellos que modulan la acción de una proteina de fusión de albumina del presente invento. Un ejemplo de tal ensayo comprende la combinación de una célula de fibroblasto mamífero, una proteina de fusión de albúmina del presente invento, y el compuesto a ser seleccionado y timidina 3[H] bajo condiciones de cultivo celular donde la célula de fibroblasto normalmente proliferaría. Un ensayo de control puede ser realizado en la ausencia del compuesto a ser seleccionado y comparado con la cantidad de proliferación de fibroblastos en la presencia del compuesto para determinar si el compuesto estimula la proliferación al determinar la absorción de timidina 3[H] en cada caso. La cantidad de proliferación de células de fibroblastos es medida mediante cromatografía de centelleo liquido la cual mide la incorporación de timidina 3[H]. Tanto los compuestos agonistas como antagonistas se pueden identificar mediante este procedimiento.

En otro método, una célula mamífera o preparación de membrana expresando un receptor para el componente de proteína Terapéutica de una proteína de fusión del invento es incubado

con una proteína de fusión rotulada del presente invento en presencia del compuesto. La capacidad del compuesto para aumentar o bloquear esta interacción luego se podría medir. Alternativamente, se mide la respuesta de un segundo sistema mensajero después de interacción de un compuesto a ser seleccionado y el receptor y la capacidad del compuesto para unirse al receptor y producir una segunda respuesta mensajera es medida para determinar si el compuesto es una proteína de fusión potencial. Tales segundos sistemas mensajeros incluyen pero no están limitados a ciclasa de guanilato de cAMP, canales iónicos o hidrólisis de fosfoinositida.

Todos estos ensayos anteriores se pueden utilizar como marcadores diagnósticos o prognósticos. Las moleculas descubiertas utilizando estos ensayos se pueden utilizar para tratar enfermedad o para causar un resultado particular en un paciente (ej., crecimiento de vasos sanguineos) al activar o inhibir la proteína de fusión/molecula. Además, los ensayos pueden descubrir agentes que pueden inhibir o aumentar la producción de las proteínas de fusión de albúmina del invento a partir de tejidos o células manipulados de manera apropiada.

Por lo tanto, el invento incluye un método para identificar compuestos que se unen a una proteína de fusión de albúmina del invento comprendiendo los pasos de: (a) incubar un compuesto de unión candidato con una proteína de fusión de albúmina del presente invento; y (b) determinar si la unión ha ocurrido. Además, el invento incluye un método para identificar agonistas/antagonistas comprendiendo los pasos de: (a) incubar un compuesto candidato con una proteína de fusión de albúmina del presente invento, (b) probar la actividad biológica, y (b) determinar si la actividad biológica de la proteína de fusión ha sido alterada.

Liberación Dirigida

En otra realización, el invento proporciona un método para liberar composiciones a células objetivo expresando un receptor para un componente de una proteína de fusión de albúmina del invento.

Como se discutió en la presente, las proteínas de fusión del invento se pueden asociar con polipéptidos heterologos, ácidos nucleicos heterólogos, toxinas o profármacos a través de interacciones hidrofóbicas, hidrofílicas, inónicas y/o covalentes. En una realización, el invento proporciona un método para la liberación específica de composiciones del invento a células mediante administración de proteínas de fusión del invento (incluyendo anticuerpos) que están asociados con polipéptidos heterologos o ácidos nucleicos. En un ejemplo, el invento proporciona un método para liberar una proteína Terapéutica en la célula objetivo. En otro ejemplo, el invento suministra un método para liberar un ácido nucleico de hebra simple (ej., antisentido o ribozimas) o ácido nucleico de hebra doble (ej., ADN que se puede integrar en el genoma de la célula o se puede replicar episomalmente y que se puede transcribir) en la célula objetivo.

En otra realización, el invento proporciona un método para destrucción específica de células (ej., la destrucción de células tumorales) mediante la administración de una proteína de fusión de albúmina del invento (ej., polipétidos del invento o anticuerpos del invento) en asociación con toxinas o profármacos citotóxicos.

Por "toxina" se entiende compuestos que fijan y activan sistemas efectores citotóxicos endógenos, radioisotopos, holotoxinas, toxinas modificadas, subunidades catalíticas de toxinas, o cualquier molecula o enzimas normalmente no presentes en o sobre la superficie de una célula que bajo condiciones definidas producen la muerte de la célula. Las toxinas que se pueden utilizar según los métodos del invento incluyen, pero no están limitadas a, radioisotopos conocidos en el estado de la técnica, compuestos tales como, por ejemplo, anticuerpos (o porciones que contienen fijación de complemento de los mismos) que se unen a un sistema efector citotóxico endógeno inherente o inducido, timidina quinasa, endonucleasa, ARNse, toxina alfa, ricina, abrin, *Pseudomonas* etotoxina A, toxina de difteria, saporina, momordina, gelonina,

proteina antiviral de minutisa, alfa-sarcina y toxina de colera. Por "profármaco citotóxico" se entiende un compuesto no tóxico que es convertido por una enzima, normalmente presente en la célula, en un compuesto citotóxico. Los profármacos citotóxicos que se pueden utilizar según los métodos del invento incluyen, pero no están limitados a derivados de glutamilo de agente alquilante de mostaza de ácido benzoico, derivados de fosfato de etoposide o mitomicina C, arabinosida de citosina, daunorubisina, y derivados de fenoxiacetamida de doxorubicina.

Clasificación de Farmacos

Se contempla además el uso de proteínas de fusión de albúmina del presente invento, o los polinucleotidos que codifican estas proteínas de fusión, para clasificar moleculas que modifican las actividades de la proteina de fusión de albúmina del presente invento o proteínas que corresponden a la porción de proteína Terapéutica de la protéina de fusión de albúmina. Tal método incluiría poner en contacto la proteína de fusión con un compuesto seleccionado sospechoso por tener actividad antagonista o agonista, y probar la actividad de la proteina de fusión después de la unión.

Este invento es particularmente útil para clasificar compuestos terapeuticos mediante el uso de proteinas de fusión de albúmina del presente invento, o unión de fragmentos de los mismos, en cualquiera de una variedad de técnicas de clasificación de fármacos. La proteína de fusión de albumina empleada en tal prueba puede ser fijada a una soporte sólido, expresado sobre una superficie celular, libre en solución, o localizada de manera intracelular. Un método de clasificación de fármacos utiliza células huesped eucarioticas o procarioticas las cuales son transformadas de manera estable con ácidos nucleicos recombinantes expresando la proteína de fusión de albúmina. Los fármacos son clasificados contra tales células transformadas o sobrenadantes obtenidos a partir de cultivar tales células, en ensayos de unión competitiva. Uno puede medir, por ejemplo, la fórmulación de complejos entre el agente probado y la proteína de fusión de albúmina del presente invento.

Por lo tanto, el presente invento proporciona métodos para clasificar fármacos u otros agentes los cuales afectan las actividades mediadas por las proteinas de fusión de albumina del presente invento. Estos métodos comprenden poner en contacto tal agente con una proteína de fusión de albúmina del invento o un fragmento de la misma y probar la presencia de un complejo entre el agente y la proteína de fusión de albúmina o fragmento de la misma, mediante métodos bien conocidos en el estado de la técnica. En tal ensayo de unión competitiva, los agentes para clasificar tipicamente son rotulados. Después de incubación, el agente libre es separado de aquel presente en la forma unida, y la cantidad de rotulo libre y sin formar complejo es una medida de la capacidad de un agente particular para unirse a la proteína de fusión de albúmina del presente invento.

Otra técnica para clasificación de fármacos proporciona clasificación de alto rendimiento para compuestos con afinidad de unión apropiada a una proteína de fusión de albúmina del presente invento, y se describe en detalle en la solicitud de Patente Europea 84/03564, publicada en Septiembre 13 de 1984, la cual es incorporada en la presente como referencia. Brevemente declarado, grandes números de diferentes compuestos de prueba de péptidos pequeños son sintetizados sobre un sustrato sólido, tal como pasadores plásticos o alguna otra superficie. Los compuestos de prueba peptidicos se hacen reaccionar con una proteína de fusión del albúmina del presente invento y se lavan. Luego los péptidos unidos son detectados mediante métodos bien conocidos en el estado de la técnica. La proteína de fusión de albúmina purificada puede ser esparcida directamente sobre placas para utilizar en las técnicas de clasificación de fármacos antes mencionadas. Además, se pueden utilizar anticuerpos no neutralizantes para capturar el péptido e inmovilizar este sobre el soporte sólido.

Este invento también contempla el uso de ensayos de clasificación de fármacos competitivos en los cuales los anticuerpos neutralizantes capaces de unirse a una proteína fusión de albúmina del presente invento específicamente compiten con un compuesto de prueba para unión a la proteína de fusión de albúmina o fragmento de la misma. En esa forma, los anticuerpos son utilizados para detectar la presencia de cualquier péptido que comparte uno más epítopes antigénicos con una proteína de fusión de albúmina del invento.

Unión de Péptidos y Otras Moleculas

El invento también contempla métodos de clasificación para identificar polipéptidos y no polipeptídicas que fijan proteínas de fusión de albúmina del invento, y las moléculas de unión identificadas así. Estas moleculas de unión son útiles, por ejemplo, como agonistas y antagonistas de las proteínas de fusión de albúmina del invento. Tales agonistas y antagonistas se pueden utilizar, según el invento, en las realizaciones terapéuticas descritas en detalle, más adelante.

Este método comprende los pasos de: poner en contacto una proteína de fusión de albúmina del invento con una pluralidad moléculas; e identificar una molécula que se une a la proteína de fusión de albúmina.

El paso de poner en contacto la proteína de fusión de albúmina del invento con la pluralidad de moléculas se puede realizar en un número de formas. Por ejemplo, se puede contemplar inmovilización de la proteína de fusión de albúmina sobre un soporte sólido y llevar una solución de la pluralidad de moleculas en contacto con los polipéptidos inmovilizados. tal procedimiento sería parecido a un proceso cromatográfico de afinidad, con la matriz de afinidad comprendida de la proteína de fusión de albúmina inmovilizada del invento. Las moleculas con una afinidad selectiva para la proteína de fusión de albúmina luego se pueden purificar mediante selección de afinidad. La naturaleza del soporte sólido, proceso para fijación de la proteína de fusión de albúmina al soporte sólido, solvente, y condiciones de la selección o aislamiento de afinidad son ampliamente convencionales y bien conocidas por aquellos con experiencia común en el estado de la técnica.

Alternativamente, se puede separar una pluralidad de polipéptidos en fracciones sustancialmente separadas comprendiendo un subgrupo de o polipéptidos individuales. Por ejemplo, se puede separar la pluralidad polipéptidos mediante electroforesis de gel, cromatografía en columna, o un método similar conocido por aquellos con experiencia común para la separación de polipéptidos. Los polipéptidos individuales también se pueden producir mediante una célula huesped transformada en forma tal para ser expresada sobre o cerca de su superficie exterior (ej., un fago recombinante). Los aislados individuales luego se pueden "examinar" mediante una proteína de fusión de albúmina del invento, opcionalmente en presencia de un inductor se debe requerir para expresión, para determinar si cualquier interacción de afinidad selectiva toma lugar entre la proteína de fusión de albúmina y el clon individual. Antes de poner en contacto la proteína de fusión de albúmina con cada fracción comprendiendo polipéptidos individuales, los polipéptidos podrían primero ser transferidos hacia un soporte sólido por conveniencia adicional. Tal soporte sólido simplemente puede ser una pieza de membrana el filtro, tal como una hecha de nitrocelulosa o nailon. En esta forma, los clones positivos pueden ser identificados a partir de una colección de células huesped transformadas de una biblioteca de expresión, la cual alberga un constructo de ADN que codifica un polipéptido con una afinidad selectiva para una proteína de fusión de albúmina del invento. Además, la secuencia de aminoácidos del polipéptido con una afinidad selectiva para una proteína de fusión de albúmina del invento se puede determinar directamente mediante medios convencionales o la secuencia codificadora del ADN que codifica el polipéptido puede ser frecuentemente determinada de manera más conveniente. Luego la secuencia primaria se puede deducir a partir de la

correspondiente secuencia de ADN. Si la secuencia de aminoácidos se va a determinar a partir del polipéptido mismo, se pueden utilizar técnicas de microsecuenciamiento. La técnica de secuenciamiento puede incluir espectroscopia de masa.

En ciertas situaciones, puede ser deseable lavar cualquier polipéptido no unido desde una mezcla de una proteína de fusión de albúmina del invento y la pluralidad de polipéptidos antes de intentar determinar o detectar la presencia de una interacción de afinidad selectiva. Tal paso de lavado puede ser particularmente deseable cuando la proteína de fusión de albúmina del invento o la pluralidad de polipéptidos son unidos a un soporte sólido.

La pluralidad de moléculas proporcionadas según este método pueden ser proporcionadas por la via de bibliotecas de diversidad, tal como bibliotecas peptídicas o no peptídicas aleatorias o combinatoriales las cuales se pueden clasificar para moleculas que especificamente unen una proteína de fusión de albúmina del invento. Se conocen muchas bibliotecas en el estado de la técnica que se pueden utilizar, ej., bibliotecas quimicamente sintetizadas, recombinantes (ej., bibliotecas de presentación de fagos), y bibliotecas basadas en translación *in vitro*. Ejemplos de bibliotecas quimicamente sintetizadas son descritos en Fodor., Science 251:767-773 (1991); Houghten, Nature 354:84-86 (1991); Lam, Nature 354:82-84 (1991); Medynski, Bio/Technology 12:709-710 (1994); Gallop, J. Medicinal Chemistry 37(9):1233-1251 (1994); Ohlmeyer, Proc. Natl. Acad. Sci. USA 90:10922-10926 (1993); Erb, Proc. Natl. Acad. Sci. USA 91:11422-11426 (1994); Houghten, Biotechniques 13:412 (1992); Jayawickeme, Proc. Natl. Acad. Sci. USA 91:1614-1618 (1994); Salmon, Proc. Natl- Acad. Sci. USA 90:11708-11712 (1993); Publicación PCT No. WO 93/20242; y Brenner y Lerner, Proc. Natl. Acad. Sci. USA 89:5381-5383 (1992).

Ejemplos de bibliotecas de presentación de fagos son descritos en Scott. Science 249:386-390 (1990); Devlin, Science, 249:404-406 (1990); Christian, 1992, J. Mol. Biol. 227:711-718 1992); Lenstra, J. Immnunol. Meth. 152:149-157 (1992); Kay, Gene 128:59-65 (1993); y Publicación PCT No. WO94/18318 registrada en Agosto 18 de 1994.

Las bibliotecas basadas en traslación *in vitro* incluyen pero no están limitadas a aquellas descritas en la Publicación PCT No. WO 91/05058 con fecha de Abril 18 de 1991; y Mattheakis, Proc. Natl. Sci. USA 91:9022-9026 (1994).

A manera de ejemplos de bibliotecas no peptídicas una biblioteca de benzodiazepina (ver ej., Bunin, Proc. Natl. Acad. Sci. USA 91:4708-4712 (1994) se puede adaptar para utilizar. También se pueden utilizar bibliotecas peptoides (Simon, Proc. Natl. Acad. Sci. USA 89:9367-9371 (1992). Otro ejemplo de una biblioteca que se puede utilizar, en la cual las funcionalidades de amida en péptidos han sido permetiladas para generar una biblioteca combinatorial químicamente transformada, se describe por Ostresh (Proc. Natl. Acad. Sci. USA 91:11138-11142 (1994)).

La variedad de bibliotecas no peptídicas que son útiles en el presente invento es numerosa. Por ejemplo, Ecker y Crooke (Bio/Technology 13:351-360 (1995) listan benzodiazepinas, hidantoinas, piperazinedionas, bifenilos, análogos de azucar, beta-mercaptocetonas, ácidos arilacéticos, acilpiperidinas, benzopiranos, cubanos, xantinas, aminimidas, y oxazolonas como entre las especies químicas que forman la base de varias bibliotecas.

Las bibliotecas no peptídicas se pueden clasificar ampliamente en dos tipos: monómeros decorados y oligómeros. Las bibliotecas monómeras decoradas emplean una estructura de andamio relativamente simple sobre la cual se agrega una variedad de grupos funcionales. A menudo el andamio será una molecula con una actividad farmacológica útil conocida. Por ejemplo, el andamio puede ser la estructura de benzodiazepina.

Las bibliotecas oligoméricas no peptídicas utiliza un gran número de monómeros que son ensamblados juntos en formas que crean nuevas formas que dependen del orden de los monómeros. Entre las unidades monómeras que han sido utilizadas están carbamatos, pirrolinonas, y morfolinos. Peptoides, oligomeros tipo péptidos en los cuales la cadena secundaria está fijada al grupo alfa amino en cambio del alfa carbono, forman la base de otra versión de bibliotecas oligoméricas no peptídicas. La primera biblioteca oligomérica no péptida utilizó un tipo simple de monómero y por lo tanto contuvo un esqueleto de repetición. Las bibliotecas recientes han utilizado más de un monómero, dando a la bibliotecas flexibilidad agregada.

La clasificación de bibliotecas se pueden realizar mediante cualquiera de una variedad de métodos comunmente conocidos. Ver. ej., las sigueintes referencias, las cuales divulgan clasificación de bibliotecas peptídicas: Parmley et al., Adv. Exp. Med. Biol. 251:215-218 (1989); Scott et al., Science 249:386-390 (1990); Fowlkes et al., BioTechniques 13:422-427 (1992); Oldenburg et al., Proc. Natl. Acad. Sci USA 89:5393-5397 (1992); Yu et al ., Cell 76:933-945 (194); Staudt et al.,Science 241:577-580 (1988); Bock et al., Nature 355:564-466 (1992); Tuerk et al., Proc. Natl. Acad. Sci USA 89:6988-6992 (1992); Ellington et al., Nature 355:850-852 (1992); U. S.Pat. No. 5.096.815, U.S. Pat. No.5.223.409, y U.S. Pat. No. 5.198.346, all to Ladner et al; Rebar et al., Science 263:671-673 (1993); y PCT publication No. WO 94/18318.

En una realización específica, la clasificación para identificar una molecula que fija una proteína de fusión de albúmina del invento se puede realizar al poner en contacto los miembros de biblioteca con una proteína de fusión de albúmina del invento inmovilizada sobre una fase sólida y albergando los miembros de biblioteca que se unen a la proteína de fusión de albúmina. Ejemplos de tales métodos de clasificación, denominadas técnicas "de toma panorámica" son descritos a manera de ejemplo en Parmley, Gene 73:305-318 (1988); Fowlkes, BioTechniques 13:422-427 (1992); Publicación PCT No. WO 94/18318; y en referencias citadas en la presente.

En otra realización, el sistema de dos híbridos para seleccionar proteínas de interacción en levadura (Fields, Nature 340:245-246 (1989); Chien. Proc. Natl. Acad. Sci USA 88:9578-9582 (1991) se puede utilizar para identificar moleculas que especificamente se unen a polipéptidos del invento.

Donde la molecula de unión es un polipéptido, el polipéptido puede ser convenientemente seleccionado de cualquier biblioteca péptida, incluyendo bibliotecas peptídicas aleatorias, bibliotecas peptídicas combinatoriales, o bibliotecas peptídicas sesgadas. El término "sesgado" se utiliza en la presente para significar que el método de generación de la biblioteca es manipulado para restringir uno o más parámetros que gobiernan la diversidad de la colección resultante de moléculas, en este caso péptidos.

Por lo tanto, una biblioteca péptida verdaderamente aleatoria generaría una colección de péptidos en los cuales la probabilidad de encontrar un aminoácido particular en la posición dada del péptido es la misma para todos los 20 aminoácidos. Un sesgo se puede introducir en la biblioteca, sin embargo, al especificar, por ejemplo, que una lisina ocurre cada quinto aminoácido o que las posiciones 4, 8, y 9 de una biblioteca de decapéptido serán fijas para incluir solo arginina. Claramente, muchos tipos de sesgos se pueden contemplar, y el presente invento no está restringido a cualquier sesgo particular. Además, el presente invento contempla tipos específicos de bibliotecas peptídicas, tales como bibliotecas peptídicas de presentación de fagos y aquellas que utilizan un constructo de ADN comprendiendo un vector fago lambda con un inserto de ADN.

Como se mencionó anteriormente, en el caso de una molecula de unión que es un polipéptido, el polipéptido puede tener entre 6 y menos de 60 residuos aminoácidos aproximadamente, preferiblemente entre 6 y 10 residuos aminoácidos aproximadamente, y lo más

preferible entre 6 y 22 residuos aminoácidos. En otra realización, un polipéptido de unión tiene en el rango de 15-100 aminoácidos, o 20-50 aminoácidos.

El polipéptido de unión seleccionado puede ser obtenido mediante síntesis química o expresión recombinante.

Otras Actividades

Una proteína fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento, se pueden emplear en el tratamiento para estimular re vascularización de tejidos isquémicos debido a varias condiciones patológicas tales como trombosis, arterioesclerosis, y otras condiciones cardiovasculares. Las proteínas de fusión de albúmina del invento y/o polinucleotidos que codifican proteínas de fusión de albúmina del invento también se pueden emplear para estimular angiogénesis y regeneración de extremidades, tal como se discutió anteriormente.

Una proteína fusión de albúmina del invento y/o polinucleotidos que codifican una proteína de fusión de albúmina del invento también se pueden emplear para tratar heridas por lesiones, quemaduras, reparación postoperativa de tejidos, y úlceras puesto que éstas son mitogénicas a varias células de orígenes diferentes, tal como células de fibroblastos y células músculo esqueléticas, y por lo tanto, facilitan la reparación o reemplazo de tejido dañado o enfermo.

Una proteína de fusión de albúmina del invento y/o polinucleotidos que codifican una proteína fusión de albúmina del invento también se pueden emplear para estimular crecimiento neuronal y para tratar y prevenir daño neuronal el cual ocurre en ciertos trastornos neuronales o condiciones neuro-degenerativas tal como enfermedad de Alzheimer, enfermedad de Parkinson, y complejo relacionado con SIDA. Una proteína de fusión de albúmina del invento y/o polinucleotidos que codifican una proteína de fusión de albúmina del invento puede tener la capacidad para estimular crecimiento de condrocitos, y por lo tanto, se pueden emplear para aumentar la regeneración ósea y periodontal y ayudar en trasplantes de tejidos o injertos óseos.

Una proteína de fusión de albúmina del invento y/o polinucleotidos que codifican una proteína de fusión de albúmina del invento también se puede emplear para prevenir envejecimiento de piel por quemadura solar mediante estimulación de crecimiento de queranocitos.

Una proteína de fusión de albúmina del invento y/o polinucleotidos que codifican una proteína de fusión de albúmina del invento también se puede emplear para prevenir pérdida de cabello. A lo largo de las mismas líneas, una proteína de fusión de albúmina del invento y/o polinucleotido que codifica una proteína de fusión de albúmina del invento se pueden emplear para estimular crecimiento y diferenciación de células hematopoyeticas y células de médula ósea cuando se utilizan en combinación con otras citoquinas.

Una proteína de fusión de albúmina del invento y/o polinucleotidos que codifican una proteína de fusión de albúmina del invento también se pueden emplear para mantener órganos antes de trasplantes o para soportar cultivos de células de tejidos primarios. Una proteína de fusión de albúmina del invento y/o polinucleotidos que codifican una proteína de fusión de albúmina también se pueden emplear para inducir tejido de origen mesodérmico para diferenciar en embriones tempranos.

Una proteína fusión de albúmina del invento y/o polinucleotido que codifica una proteína de fusión de albúmina del invento también puede aumentar o disminuir la diferenciación o proliferación de células madre enbrionarias, además de, como se discutió anteriormente, linaje hematopoyetico.

Una proteína de fusión de albúmina del invento y/o polinucleotidos que codifican una proteína de fusión de albúmina del invento también se pueden utilizar para modular características mamíferas, tales como estatura, peso, color de cabello, color de ojos, piel, porcentaje de tejido adiposo, pigmentación, tamaño, y forma (ej., cirugía cosmética). De manera similar, una proteína de fusión de albúmina del invento y/o polinucleotidos que codifican una proteína fusión de albúmina del invento se pueden utilizar para modular metabolismo mamífero que afecta metabolismo, catabolismo, procesamiento, utilización, y almacenamiento de energía.

Una proteína de fusión de albúmina del invento y/o polinucleotidos que codifican una proteína de fusión de albúmina del invento se pueden utilizar para cambiar el estado físico o estado mental de un mamífero al influenciar los bioritmos, rítmos cardiacos, depresión (incluyendo trastornos depresivos), tendencia a violencia, tolerancia de dolor, capacidades reproductivas (preferiblemente mediante actividad tipo Activin o Inhibin), niveles hormonales o endocrinos, apetito, líbido, memoria, estrés, u otras cualidades cognoscitivas.

Una proteína de fusión de albúmina del invento y/o polinucleotidos que codifican una proteína de fusión de albúmina también se pueden utilizar como un aditivo o preservativo de alimentos, para aumentar o disminuir capacidades de almacenamiento, contenido graso, lípidos, proteínas, carbohidratos, vitaminas, minerales, cofactores u otros componentes nutricionales.

Las aplicaciones citadas anteriormente tienen usos en una amplia variedad de huéspedes. Tales huéspedes incluyen, pero no están limitados a humano, murino, conejo, cabra, conejillo de indias, camello, caballo, ratón, rata, hamster, cerdo, micro-cerdo, pollo, cabra, vaca, oveja, perro, gato, primate no humano, y humano. En realizaciones específicas, el huésped es un ratón, conejo, cabra, conejillo de indias, pollo, rata, hamster, cerdo, oveja, perro o gato. En realizaciones preferidas, el huésped es un mamífero. En realizaciones más preferidas, el huésped es un humano.

Habiendo descrito generalmente el invento, lo mismo se entenderá de manera más fácil como referencia a los siguientes ejemplos, los cuales se suministran a manera de ilustración y no pretenden ser limitantes.

Sin descripción adicional, se cree que alguien con experiencia común en el estado de la técnica puede, utilizando la anterior descripción y los siguientes ejemplos ilustrativos, hacer y utilizar las alteraciones detectadas en el presente invento y practicar los métodos reivindicados. Los siguientes ejemplos practicables por lo tanto, especificamente señalan realizaciones preferidas del presente invento, y no se consideran como limitantes en forma alguna del resto de la divulgación.

EJEMPLOS

EJEMPLO 1: Generación de pScNHSA y pScCHSA

Los vectores pScNHSA (Deposito ATCC No. PTA-3279) y pScCHSA (Deposito ATCC No. PTA-3276) son derivados de pPPC0005 (Depósito ATCC No. PTA-3278) y se utilizan como vectores de clonación en los cuales los polinucleotidos que codifican una proteína terapéutica o fragmento o variante de la misma son insertados adyacentes a y en marco de traslación con polinucleotidos que codifican albúmina sérica humana "HSA". pScCHSA se puede utilizar para generar fusiones proteína terapéutica-HSA, mientras pScNHSA se puede utilizar para generar fusiones de HSA-proteína Terapéutica.

Generación de pScCHSA: fusión de albúmina con la fracción de albúmina C-terminal a la porción terapéutica.

Un vector para facilitar clonación de ADN que codifica una proteína terapéutica N-terminal a ADN que codifica la proteína de albúmina madura fue hecho al alterar la secuencia de

ácidos nucleicos que codifica el péptido de señal HSA quimérico en pPPC0005 para incluir los sitios de restricción *Xho* I y *Cla* I.

Primero, los sitios *Xho* I y *Cla* I inherentes a pPPC0005 (localizados 3' de la secuencia terminadora ADH1) fueron elimitados al digerir pPPC0005 con *Xho* I y *Cla* I, llenando los extremos pegajosos con polimerasa T4 ADN, y religando los extremos romos para crear pPPC0006.

Segundo, los sitios de restricción *Xho* I y *Cla* I fueron diseñados en la secuencia de ácidos nucleicos que codifica el péptido de señal de HSA (una química de la HSA guía y un sitio kex2 desde el factor de conjugación alfa, "MAF") en pPPC0006 utilizando dos rondas de PCR. En la primera ronda de PCR, la amplificación con cebadores mostrados como SEQ ID NO:36 y SEQ ID NO:37 fue realizada. El cebador cuya secuencia es mostrada como SEQ ID NO:36 comprende una secuencia de ácidos nucleicos que codifica parte de la secuencia de péptidos de señal de HSA, un sitio kex2 desde la secuencia guía de factor de conjugación alfa, y parte del amino-término de la forma madura de HSA. Cuatro mutaciones de punto fueron introducidas en la secuencia, creando los sitios *Xho* I y *Cla* I encontrados en la unión del péptido de señal quimérico y la forma madura de HSA. Estas cuatro mutaciones están subrayadas en la secuencia mostrada a continuación. En pPPC0005 los nucleotidos en estas cuatro posiciones desde 5' a 3' son T, G, T, y G.

5`-
GCCTCGAGAAAAGAGATGCACACAAGAGTGAGGTTGCTCATCGATTTAAAGATTTTG
GG-3`(SEQ ID NO:36) y 5`-
AATCGATGAGCAACCTCACTCTTGTGTGCATCTCTTTTCTCGAGGCTCCTGGAAGC-
3´(SEQ ID NO:37).

Una segunda ronda de PCR fue luego realizada con un cebador de flanqueador corriente arriba, 5'-TACAAACTTAAGAGTCCAATTAGC-3' (SEQ ID NO:38) y un cebador de flanco corriente abajo 5'-CACTTCTCTAGAGTGGTTTCATATGTCTT-3' (SEQ ID NO:39). Luego el producto PCR resultante fue purificado y digerido con *Afl* II y *Xba* I y ligado en los mismos sitios en pPPV0006 creando pScCHSA. El plásmido resultante tienen sitios *Xho* I y *Cla* I diseñados en la secuencia de señal. La presencia del sitio *Xho* I crea un cambio de aminoácido simple en el extremo de la secuencia de señal desde LDKR a LEKR. El cambio de D a E no estará presente en el plásmido de expresión de proteína de fusión de albúmina final cuando una secuencia de acidos nucleicos comprendiendo un polinucleotido que codifica la porción terapéutica de la proteína de fusión de albúmina con un sitio *5' Sal* I (el cual es compatible con el sitio *Xho* I) y un sitio 3' *Cla* I es ligado a los sitios *Xho* I y *Cla* I de PScCHSA. La ligación de *Sal* I a *Xho* I restaura la secuencia aminoácida original de la secuencia de péptidos de señal. ADN codificando la porción Terapeutica de la proteína de fusión de albúmina se puede insertar después del sitio Kex2 (Kex2 se divide después de la secuencia de aminoácidos dibásica KR en el extremo del péptido de señal) y antes del sitio *Cla* I

Generación de pScNHSA: fusión de albúmina con la fracción de albúmina N-terminal a la porción terapéutica.

Un vector para facilitar clonación de ADN que codifica una porción de proteína Terapeutica C-terminal a ADN que codifica la proteína de albúmina madura, se preparó agregando tres sitios de restricción de ocho pares base a pScCHSA. Los sitios de restricción *Asc* I, *Fse* I, *Pme* I fueron agregados entre los sitios *Bsu36* I y *Hind* III en el extremo de la secuencia de ácidos nucleicos codificando la proteina HSA madura. Esto se logro a través del uso de dos cebadores sintéticos complementarios conteniendo los sitios de restricción *Asc* I, *Fse* I, *Pme* I subrayados (SEQ ID NO:40 y SEQ ID NO:41).

5'-
AAGCTGCCTTAGGCTTATAATAAGGCGCCGGCCGGCCTTTTAAACTAAGCTTAATTC
T-3' (SEQ ID NO:40) y 5-
AGAATTAAGCTTAGTTTAAACGGCCGGCCGGCGCGCCTTATTATAAGCCTAAGGCAG
CTT-3'(SEQ ID NO:41).. Estos cebadores fueron endurecidos y digeridos con *Bsu36 I* y *Hind* III y ligados en los mismos sitios en pScCHSA creando pScNHSA.

EJEMPLO 2: Generación de Constructo General para Transformación de Levadura.

Los vectores pScNHSA y pScCHSA se pueden utilizar como vectores de clonación en los cuales los polinucleotidos que codifican una proteína terapeutica o fragmento o variante de la misma son insertados adyacentes a polinucleotidos que codifican albúmina sérica humana "HSA". pScCHSA se utiliza para generar fusiones de proteína terapéutica-HSA, mientras pScNHSA se puede utilizar para generar fusiones de HSA-proteína terapéutica.

Generación de constructos de fusión de albúmina comprendiendo productos de fusión de HSA-proteína terapéutica.

ADN que codifica una proteína Terapéutica (ej., secuencias conocidas en SEQ ID NO:X o conocidas en el estado de la técnica) pueden ser PCR amplificadas utilizando cebadores que facilitan la generación de un constructo de fusión (ej., al agregar sitios de restricción, codificando fusiones sin unión, codificando secuencia enlazadoras, etc.) Por ejemplo, alguien con experiencia en el estado de la técnica podría diseñar un cebador 5' que agrega polinucleotidos que codifican los últimos cuatro aminoácidos de la forma madura de HSA (y conteniendo el sitio *Bsu361*) sobre el extremo 5' del ADN que codifica una proteína Terapeutica; y un cebador 3' que agrega un codon de TOPE y sitios de clonación apropiados sobre el extremo 3' de la secuencia codificadora de proteína Terapeutica. Por ejemplo, el cebador delantero utilizado para amplificar ADN que codifica una proteína terapéutica puede tener la secuencia, 5'-aagctGCCTTAGGCTTA(N)15-3' (seq id no:42) donde la secuencia subrayada es un sitio *Bsu361*, los nucleotidos en mayuscula codifican los últimos cuatro aminoácidos de la proteína HSA madura (ALGL), y (N)15 es es identico a los primeros quince nucleotidos que codifican la proteína terapéutica de interes. De manera similar, el cebador inverso utilizado para amplificar ADN que codifica una proteína Terapéutica puede tener la secuencia, 5'-
GCGCGCGTTTAAACGGCCGGCCGGCGCGCCTTATTA(N)15-3' (SEQ ID NO:43) donde la secuencia en cursiva es un sitio *Pme* I, y la secuencia con doble subrayado es un sitio *Fse* I, la secuencia subrayada de manera simple es un sitio *Asc* I, los nucleotidos en el cuadro son el complemento inverso de dos codones de tope tamden, y (N)15 es identico al complemento inverso de los últimos 15 nucleotidos que codifican la proteína terapética de interés. Una vez amplificado el producto PCR este se puede cortar con *Bsu*361 y uno de (*Asc* I, *Fse* I, *o Pme* I) y se puede ligar en pScNHSA.

La presencia del sitio *Xho* en la secuencia guía quimérica crea un cambio aminoácido simple en el extremo de la secuencia de señal quimérica, es decir, la secuencia de señal HSA-kex2, desde LDKR (SEQ ID NO:44) a LEKR (SEQ ID NO:45).

Generación de constructos de fusión de albúmina comprendiendo productos de fusión de genes-HSA

Similar al método descrito anteriormente, el ADN que codifica una proteína terapeutica se puede amplificar por PCR utilizando los siguientes cebadores: Un cebador 5' que agrega polinucleotidos que contienen un sitio *Sal*I y que codifican los últimos tres aminoácidos de la secuencia guía HSA, DKR, sobre el extremo 5' de ADN que codifica una proteína Terapéutica; y un cebador 3' que agrega polinucleotidos que codifican los primeros pocos aminoácidos del HSA

maduro conteniendo un sitio *Cla* sobre el extremo 3' de ADN codificando una proteína terapéutica. Por ejemplo, el cebador delantero utilizado para amplificar el ADN que codifica una proteína terapéutica puede tener la secuencia, 5'-aggagcgtcGACAAAGA(N)15-3' (SEQ ID NO:46) donde la secuencia subrayada es un sitio *Sal* I, los nucleotidos en mayuscula codifican los últimos tres aminoácidos de la secuencia guía HSA (DKR), y (N)15 es identico a los primeros 15 nucleotidos que codifican la proteína terapéutica de interés. De manera similar, el cebador invertido utilizado par amplificar el ADN que codifica una proteína Terapéutica puede tener la secuencia, 5'-CTTAA*ATCGAT*GAGCAACCTCATCCTTGTGTGCATC (N)15-3'(SEQ ID NO:47) donde la secuencia en cursiva es un sitio *Cla* I, los nucleotidos subrayados son el complemento inverso del ADN que codifica los primeros 9 aminoácidos de la forma madura de HSA (DAHKSEVAH, SEQ ID NO:48), y (N)15 es identico al complemento inverso de los últimos 15 nucleotidos que codifican la proteína Terapéutica de interés. Una vez el producto PCR es amplificado, éste se puede cortar con *Sal* I y *Cla* I y ligar en pScCHSA digerido con *Xho* y *Cla* I. Una señal diferente o secuencia guía puede ser deseada, por ejemplo, "INV" invertasa (Swiss-Prot Acceso P23142), factor de conjugación alfa "MAF" (Genbank Acceso AAA18405), MPIF (Geneseq AAF82936), Fibulina B (Swiss-Prot Acceso P10909), Factor de Crecimiento tipo insulina - Proteína de Unión 4 (Swiss-Prot Acceso P22692), y permutas de la secuencia guía HSA se pueden subclonar en el vector apropiado por medio de métodos estándares conocidos en el estado de la técnica.

Generación de constructo de fusión de albúmina compatible para la expresión en levadura S. cervisiae.

El fragmento *Not I* que contiene el ADN que codifica una proteína de fusión de albúmina N-terminal o C-terminal generada a partir de pScNHSA o pScCHSA puede luego se clonado en el sitio *Not* I de pSAC35 el cual tiene un marcador seleccionable LEU2. Luego el vector resultante es utilizado en la transformación de un sistema de expresión de levadura *S. cerevisiae.*

EJEMPLO 3: Expresión General en levadura S. cerevisae.

Un vector de expresión compatible con la expresión de levadura se puede transformar en levadura *S. cerevisiae* mediante transformación de acetato de litio, electroporación, u otros métodos conocidos en el estado de la técnica y o como se describió en parte en Sambrook, Fritsch, y Maniatis. 1989. "Molecular Cloning: A Laboratoy Manual, 2da Edición", volumenes 1-3 y en Ausubel 2000. Massachusetts Hospital General y Escuela Médica de Harvard "Current Protocols in Molecular Biology", volumenes 1-4. Los vectores de expresión son introducidos en las cepas de *S. cerevisiae* DXY1, D88, o BXP10 mediante transformación, transformantes individuales se pueden cultivar, por ejemplo, durante 3 días a 30°C en 10 mL de YEPD (1% w/v de extracto de levadura, 2% w/v, peptona, 2% w/v, dextrosa), y las células se pueden recolectar en la fase estacionaria después de 60 horas de crecimiento. Los sobrenadantes son recolectados al clarificar células a 3000g durante 10 minutos.

pSAC35 (Sleep, 1990, Biotechnology 8:42 y ver Figura 3) comprende, además del marcador seleccionable LEU2, 2 um de plásmido de levadura completa para proporcionar funciones de replicación, el promotor PRB1, y la señal de terminación ADH1.

EJEMPLO 4: Purificación General de una Proteína de Fusión de Albúmina Expresada a partir de una Fusión de Albúmina en Levadura S. cerevisiae.

En realizaciones preferidas, las proteínas de fusión de albúmina del invento comprenden la forma madura de HSA fusionada a cualquiera el término N o C en la forma madura de una proteína terapeutica o porciones de la misma (ej., la forma madura de una proteina terapéutica listada en la Tabla 1, o la forma madura de una proteína terapéutica mostrada en la Tabla 2 como SEQ ID NO:Z). En una realización del invento, las proteínas de fusión de albúmina del invento

además comprenden una secuencia de señal que dirige el polipéptido de fusión naciente en las rutas secretorias del huesped utilizado para expresión. En una realización preferida, el péptido de señal codificado por la secuencia de señal es retirado, y la proteína de fusión de albúmina madura es secretada directamente en el medio de cultivo. Las proteínas de fusión de albúmina del invento preferiblemente comprenden secuencias de señal heterólogas (ej., la secuencia de señal no nativa de una proteína terapéutica particular) incluyendo, pero sin limitar a MAF, INV, Ig, Fibulina B, Clusterina, Proteína 4 de Unión de Factor de Crecimiento tipo Insulina, secuencias guía DE HSA variante incluyendo, pero sin limitar a una secuencia guía quimérica HSA/MAF, u otras secuencias de señal heterólogas conocidas en el estado de la técnica. Especialmente preferidas son aquellas secuencias de señal listadas en la Tabla 2 y/o la secuencia de señal listada en la sección "Expresión de Proteínas de Fusión" y/o "Métodos Adicionales de Producción Recombinante y Sintética de Proteinas de Fusión de Albúmina" de la especificación anterior. En realizaciones preferidas, las proteínas de fusion del invento además comprenden un residuo de metionina N-terminal. Los polinucleotidos que codifican estos polipéptidos, incluyendo fragmentos y/o variantes, también son contemplados por el invento.

Las proteínas de fusión de albúmina expresadas en levadura como se describió anteriormente son purificadas a escala pequeña sobre una columna de afinidad de péptido Dyax como sigue. Los sobrenadantes de levadura que expresan una proteína de fusión de albúmina son diafiltrados contra amortiguador de fosfato 3 mM pH 6.2, 20 mM NaCL y 0.01% Tween 20 para reducir el volumen y para remover los pigmentos. Luego la solución es filtrada a través de un dispositivo de 0.22 um. El filtrado es cargado sobre una columna de afinidad péptida Dyax. La columna es eluida con amortiguador 100 mM Tris/HCl, pH 8.2. Las fracciones máximas que contienen la proteína son recolectadas y analizadas sobre SDS-PAGE después de concentrar cinco veces.

Para purificación a gran escala, se puede utilizar el siguiente método. El sobrenadante en exceso de 2 L es diafiltrado y concentrado a 500 mL en 20 mM Tris/HCl pH 8.0. La solución de proteína concentrada es cargada sobre una columna de Flujo Rapido de DEAE-Sefarosa de 50 mL pre-equilibrada, la columna es lavada, y la proteína es eluida con un gradiante lineal de NaCl de 0 a 0.4 M NaCl en 20 mM de Tris/CHl, pH 8.0. Aquellas fracciones conteniendo la columna son agrupadas, ajustadas a pH 6.8 con fosfato de sodio 0.5 M (NaH2PO4). Una concentración final de 0.9 M (NH4)2SO4 es agregada a la solución de proteínas y la solución completa es cargada sobre una columna Butyl650S de 50 mL pre-equilibrada. La proteína es eluida con un gradiente lineal de sulfato de amonio (0.9 a 0 M (NH4)2SO4). Aquellas fracciones con la proteína de fusión son nuevamente agrupadas, diafriltradas contra 10 mM de Na2HPO4/amortiguador de ácido cítrico pH 5.75, y cargadas sobre una columna de Flujo Rapido de SP-Sefarosa pre-equilibrada de 50 mL. La proteína es eluida con un gradiente lineal de NaCl de 0 a 0.5 M. Las fracciones que contienen la proteína de interés son combinadas, el amortiguador es cambiado a 10 mM Na2HPO4/ácido cítrico pH 6.25 con un concentrador Amicon, la conductividad es < 2.5 mS/cm. Esta solución proteica es cargada sobre una columna de alto rendimiento de Q-Sefarosa pre-equilibrada de 15 mL, la columna es lavada, y la proteína es eluida con un gradiente lineal NaCl de 0 a 0.15 M NaCl. Luego la proteína purificada se pueden fórmular en una composición de amortiguador especifico mediante intermcambio de amortiguador.

EJEMPLO 5: Generación de Constructo General para Transfección de Células Mamíferas

Generación de constructo de fusión de albúmina para expresión en lineas celulares mamíferas

Los constructos de fusión de albúmina se pueden generar en vectores de expresión para utilizar en sistemas de cultivos de células mamíferas. El ADN que codifica una proteína terapéutica puede ser clonado N-término o C-término a HSA en un vector de expresión mamífero mediane métodos estándares conocidos en el estado de la técnica (ej., amplificación PCR, digestión de restricción, y ligación). Una vez construido el vector de expresión, la transfección hacia una sistema de expresión mamífero puede avanzar. Vectores apropiados son conocidos en el estado de la técnica incluyendo, pero sin limitar a, por ejemplo, el vetor pC4, y/o vectores disponibles en Lonza Biologics, Inc., (portsmouth, NH).

El ADN que codifica albúmina sérica humana ha sido clonado en el vector pC4 el cual es apropiado para sistemas de cultivo mamiferos, creando plásmido pC4:HSA (Deposito ATCC # PTA-3277). Este vector tiene un gen de DiHydroFolato Reductasa "DHFR" que permitirá la selección en la presencia de metotrexato.

El vector pC4:HSA es apropiado para expresión de proteínas de fusión de albúmina en células CHO. Para expresión, en otros sistemas de cultivo de células mamíferas, puede ser deseable subclonar un fragmento comprendiendo, o alternativamente constando de ADN que codifica una proteína de fusión de albúmina madura se puede subclonar en otro vector de expresión incluyendo, pero sin limitar a cualquier vector de expresión mamífero descrito en la presente.

En una realización preferida, el ADN que codifica un constructo de fusión de albúmina es subclonado en vectores proporcionados por Lonza Biologics, Inc. (Portsmouth, NH) mediante procedimientos conocidos en el estado de la técnica para expresión en células NS0.

Generación de constructos de albúmina comprendiendo productos de fusión de HSA-Proteína Terapéutica.

Utilizando pC4:HSA (ATCC Depósito #PTA-3277), se pueden generar constructos de fusión de albúmina en los cuales la porción de proteína Terapéutica es C-terminal a la secuencia de albúmina madura. Por ejemplo, se puede clonar ADN que codifica una proteína terapéutica de fragmento o variante de la misma entre los sitios de restricción *Bsu* 361 y *Asc* I del vector. Cuando se clona a *Bsu* 361 y *Asc* I, se puede emplear el mismo diseño de cebador utilizado para clonar al sistema de vector de levadura (SEQ ID NO:42 y 43) (ver Ejemplo 2).

Generación de constructos de fusión de albúmina comprendiendo productos de fusión de gen-HSA.

Utilizando pC4:HSA (Depósito ATCC # PTA-3277), se pueden generar constructos de fusión de albúmina en los cuales una porción de proteína Terapéutica es N-terminal clonada a la secuencia de albúmina madura. Por ejemplo, se puede clonar ADN que codifica una proteína Terapéutica que tiene su propia secuencia de señal entre los sitios *Bam* HI (o *Hind* III) y *Cla* de pC4:HSA. Cuando se clona a uno u otro sitio *Bam* HI o *Hind* III, es preferible incluir una secuencia Kozak (CCGCCACCATG, SEQ ID NO:49) antes del codon de inicio translacional del ADN que codifica la proteína Terapéutica. Si una proteína Terapéutica no tienen un secuencia de señal, el ADN que codifica esa proteína Terapéutica se puede clonar entre los sitios *Xho* I y *Cla* I de pC4:HSA. Cuando se utiliza el sitio *Xho* I, los siguientes cebadores PCR de ejemplo 5' (SEQ ID NO:50) y 3'(SEQ ID NO:51) se pueden utilizar:

5'-CCGCCGCTCGAGGGGTGTGTTTCGTCGA$(N)_{18}$-3'(SEQ ID NO:50)

5'-AGTCCCATCGATGAGCAACCTCACTCTTGTGTGCATC$(N)_{18}$-3'(SEQ ID NO:51)

En el cebador 5' (SEQ ID NO:50), la secuencia subrayada es un sitio *Xho* I y el ADN después del sitio *Xho* I codifica los ñultimos siete aminoácidos de la secuencia guía de albúmina

sérica humana natural. En SEQ ID NO:50, "(N)18" es ADN idéntico a los primeros 18 nucleotidos que codifican la proteína Terapéutica de interés. En el cebador 3' (SEQ ID NO:51), la secuencia subrayada es un sitio *Cla* I; y el sitio *Cla* I y el ADN después de este son el complemento inverso del ADN que codifica los primeros 10 aminoácidos de la proteína HSA madura (SEQ ID: NO:1). En SEQ ID:51 "(N)18" es el complemento inverso de ADN que codifica los últimos 18 nucleotidos que codifican la proteína terapéutica de interés. Utilizando estos dos observadores, se puede amplificar PCR la proteína de interés, purificar el producto PCR, digerir éste con las enzimas de restricción *Xho* I y *Cla* I y clonarlo en los sitios *Xho* I y *Cla* I en el vector pC4:HSA.

Si se desea una secuencia guía alternativa, la secuencia guía de albúmina nativa se puede reemplazar con la guía de albúmina quimérica, es decir, el péptido de señal HSA-kex2, o una guía alternativa mediante métodos estándares conocidos en el estado de la técnica. (Por ejemplo, alguien con experiencia en el estado de la técnica podría amplificar mediante PCR de manera común una guía alterna y subclonar el producto PCR en un constructo de fusión de albúmina en lugar de la guía de albúmina mientras se mantiene el marco de lectura).

EJEMPLO 6: Expresión General en Líneas Celulares Mamíferas.

Un constructo de fusión de albúmina generado en un vector de expresión compatible con expresión en lineas celulares mamíferas se puede transfectar en líneas celulares apropiadas mediante precipitación de fosfato de calcio, lipofectamina, electroporación, u otros métodos de transfección conocidos en el estado de la técnica y/o como se describe en Sambrook, Fritsch, y Manitis. 1989. "Molecular Cloning: A Laboratory Manual, 2da edición y en Ausubel 2000. Hospital General de Massachusetts y Escuela de Medicina de Harvard "Current Protocols in Molecular Biology", volumenes 1-4. Luego las células transfectadas son seleccionadas mediante la presencia de un agente de selección determinado por el marcador seleccionable en el vector de expresión.

El vector de expresión pC4 (Acceso ATCC No. 209646) es un derivado del plásmido pSV2-DHFR (Acceso ATCC No. 37146). pC4 contiene las Repeticiones Terminales Largas de promotor fuerte "LTR" del virus de Sarcoma de Rous (Cullen, Marzo 1985, Biología Molecular y Celular, 438-447) y un fragmento del mejorador de CitoMegaloVirus "CMV" (Boshart, 1985, Cell 41: 521-530). El vector también contiene el 3' intron, la señal de poliadenilación y terminación del gen de preproinsulina de rata, y el gen DHFR de ratón bajo control del promotor temprano SV40. Las células de ovario de hamster Chino "CHO" u otras líneas celular que carecen de un gen DHFR activo son utilizadas para transfección. La transfección de un constructo de fusión de albúmina en pC4 a células CHO mediante métodos conocidos en el estado de la técnica permitirá la expresión de la proteína de fusión de albúmina en las células CHO, seguido por escisión de la secuencia guía y secreción en el sobrenadante. Luego la proteína de fusión de albúmina es purificada adicionalmente a partir del sobrenadante.

El vector de expresión pEE12.1 es proporcionado por Lonza Biologics, Inc. (Portsmouth, NH) y es un derivado de pEE6 (Stephens y Cockett, 1989, Acidos Nucleicos Res. 17:7110). Este vector comprende un promotor, aumentador y región 5'-no trasladada completa del gen Temprano Inmediato Principal del CitoMegaloVirus , "hCMV-MIE" (Publicación Internacional # WO89/01036), corriente arriba de una secuencia de interés, y un gen de sintasa de glutamina (Murphy 1991, Biochem J. 227:277-279; Bebbington, 1992, Bio/Tecnology 10:169-175; Patente Americana US 5.122.464) para propósitos de selección de células transfectadas en medio conteniendo sulfoximina de metionina selectiva. La transfección de los constructos de fusión de albúmina hecha en PEE12.1 a células NS0 (Publicación Internacional #WO86/05807) mediante métodos conocidos en el estado de la técnica permitirá la expresión de la proteina de fusión de

albúmina en células NS0, seguido por la escisión de la secuencia guía, y secreción en el sobrenadante. Luego la proteína de fusión de albúmina es purificada adicionalmente a partir del sobrenadante utilizando técnicas descritas en la presente o de otra manera conocidas en el estado de la técnica.

La expresión de una proteína de fusión de albúmina se puede analizar, por ejemplo, mediante SDS-PAGE y Western blot, análisis HPLC de fase inversa, y otros métodos conocidos en el estado de la técnica.

Líneas celulares CHO y NS0 estables transfectadas con constructos de fusión de albúmina son generadas mediante métodos conocidos en el estado de la técnica (ej., transfección de lipofectamina) y seleccionadas, por ejemplo, con 100 nM metotrexato para vectores con el gen de DiHidroFolato Reductasa "DHFR" como un marcador seleccionable o a través de crecimiento en la ausencia de glutamina. Los niveles de expresión se pueden examinar por ejemplo, mediante immunoabsorción principalmente, con un suero anti-HSA con el anticuerpo primario, o, de manera secundaria, con suero conteniendo anticuerpos dirigidos a la porción de proteína Terapéutica de una proteína de fusión de albumina dada como el anticuerpo primario.

Los niveles de expresión son examinados mediante detección de inmunosorbente con suero anti-HSA como el anticuerpo primario. Las tasas de productividad específicas son determinadas mediante ELISA en cual el anticuerpo capturado puede ser un anticuerpo monoclonal hacia la porción de proteína terapéutica de la fusión de albúmina y el anticuerpo de detección puede ser el anticuerpo monoclonal anti-HSA-biotinilado (*o viceversa*), seguido por unión de peroxidasa de rábano/estreptavidina y análisis según el protocolo del fabricante.

EJEMPLO 7: Expresión de una Proteína de Fusión de Albúmina en Células Mamíferas.

Las proteínas de fusión de albúmina del presente invento se pueden expresar en una célula mamífera. Un vector de expresión mamífero típico contiene un elemento promotor, el cual media la iniciación de transcripción de mARN, una secuencia codificadora de proteínas, y señales requeridas para terminación de transcripción y poliadenilación del trascripto. Elementos adicionales incluyen mejoradores, secuencias Kozak e intrones flanqueados por sitios donantes y aceptadores para unión de ARN. La transcripción altamente efectiva es lograda con los promotores tempranos y tardíos a partir de SV40, las repeticiones terminales largas (LTRs) a partir de retrovirus, ej., RSV, HTLVI, HIVI y el promotor temprano del citomegalovirus (CMV). Sin embargo, también se pueden utilizar elementos celulares (ej., el promotor de actina humana).

Vectores de expresión apropiados para utilizar en la práctica del presente invento incluyen, por ejemplo, vectores tales como pSVL y pMSG (Pharmacia, Uppsala, Suecia), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSport 2.0, y pCMVSport 3.0. Células huesped mamíferas que se pueden utilizar incluyen, pero no están limitadas a células Hela humanas, 293, H9, y Jurkat, células de ratón NIH3T3 y C127, células Cos 1, cos 7 y CVI, células quail QC1-3, células de ratón L y células de ovario de Hamster Chino (CHO).

Alternativamente, la proteína de fusión de albúmina se puede expresar en líneas celulares estables que contienen el polinucleotido que codifica la proteína de fusión de albúmina integrado en un cromosoma. La co-transfección con un marcador seleccionable tal como DHFR, gpt, neomicina, o higromicina permite la identificación y aislamiento de las células transfectadas.

El polinucleotido transfectado que codifica la proteína de fusión también se puede amplificar para expresar grandes cantidades de la proteína de fusión codificada. El marcador DHFR (reductasa de dihidrofolato) es útil para desarrollar líneas celulares que llevan varios cientos o aún varios miles de copias del gen de interés. (Ver, ej., Alt. J. Biol. Chem. 253:1357-

1370 (1978); Hamlin, Biochem. et Biophys Acta, 1097:107-143 (1990); Page, Biotechnology 9:64-68 (1991)). Otro marcador de selección útil es la enzima glutmina sintasa (GS) (Murphy, Biochem J. 227:277-279 (1991); Bebbington, Bio/Technology 10:169-175 (1992). Utilizando estos marcadores, las células mamíferas son desarrolladas en medio selectivo y las células con la mayor resistencia son seleccionadas. Estas líneas celulares contienen el gen integrado en un cromosoma. Las células de ovaro de hamster chino (CHO) y NSO a menudo son utilizadas para la producción de proteínas.

Derivados del plásmido pSV2-dhfr (Acceso ATCC No. 37146), los vectores de expresión pC4 (Acceso ATCC No. 209646) y pC6 (Acceso ATCC No. 209647) contienen el promotor fuerte (LTR) del Virus de Sarcoma Rous (Cullen, Biología Molecular y Celular, 438-447 (Marzo, 1985)) más un fragmento del aumentador CMV (Boshart, Cell 41:521-530 (1985)). Múltiples sitios de clonación, ej., con los sitios de escisión restricción enzimática BamHI, XbaI y XBaI y Asp718, facilitan la clonación del gen de interés. Los vectores también contienen el intron 3', la señal de poliadenilación y terminación del gen de preproinsulina de rata, y el gen DHFR de ratón bajo control del promotor temprano SV40.

Especificamente, el plásmido pC6, por ejemplo, es digerido con enzimas de restricción apropiadas y luego desfosforilado utilizando fosfatos intestinales de ternero mediante procedimientos conocidos en el estado de la técnica. Luego el vector es aislado a partir de gel de agarosa al 1%.

Un polinucleotido que codifica una proteína de fusión de albúmina del presente invento es generado utilizando técnicas conocidas en el estado de la técnica y este polinucleotido es amplificado utilizando tecnología PCR conocida en el estado de la técnica. Si se utiliza una secuencia de señal de ocurrencia natural para producir la proteína de fusión del presente invento, el vector no necesita un segundo péptido de señal. Alternativamente, si no se utiliza una secuencia de señal de ocurrencia natural para producir la proteína de fusión del presente invento, el vector se puede modificar para incluir una secuencia de señal heteróloga. (Ver, ej., Publicación Internacional No. WO 96/34891.)

El fragmento amplificado que codifica la proteina de fusión del invento es aislado a partir de un gel de agarosa al 1% utilizando un kit comercialmente disponible ("Geneclean," BIO 101 Inc. La Jolla, Ca.). Luego el fragmento es digerido con enzimas de restricción apropiadas y nuevamente purificado sobre un gel de agarosa al 1%.

El fragmento amplificado que codifica la proteina de fusión de albúmina del invento luego es digerido con la misma enzima de restricción y purificado sobre gel de agarosa al 1%. El fragmento aislado y el vector desfosforilado luego son ligados con ligasa T4 ADN. Luego células de *E. coli* HB101 o XL-1 Blue son transformadas y se identifican las bacterias que contienen el fragmento insertado en el plásmido pC6 utilizando, análisis de enzimas de restricción.

Células de ovario de hamster Chino que carecen de un gen DHFR activo son utilizadas para trasfección. Cinco ug del plásmido de expresión pC6 o pC4 es cotransfectado con 0.5 ug del plásmido pSVneo utilizando lipofectina (Felgner, anteriormente). El plásmido pSV2-neo contiene un marcador seleccionable dominante, el gen neo de Tn5 que codifica una enzima que confiere resistencia a un grupo de antibioticos incluyendo G418. Las células son sembradas en marcador alfa minus MEM suplementadas con 1 mg/ml G418. Después de 2 días, las células son triptinizadas y sembradas en placas de clonación de hibridoma (Greiner, Alemania) en alfa minus MEM suplementado con 10, 25, o 50 ng/ml de metotrexato más 1 mg/ml de G418. Después de 10-14 días aproximadamente los clones simples son triptinizados y luego sembrados en platos petri de 6 pozos o frascos de 10 ml utilizando concentraciones diferentes de metotrexato (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Los clones creciendo a concentraciones mayores de

metotrexato luego son transferidos a nuevas placas de 6 pozos conteniendo aún concentraciones mayores de metotrexato (1 uM, 2 uM, 5 uM, 10 mM, 20 mM). El mismo procedimiento se repite hasta obtener clones que crecen a una concentración de 100 - 200 uM. La expresión de la proteína de fusión deseada es analizada, por ejemplo, mediante SDS-PAGE y Western blot o mediante análisis HPLC de fase inversa.

EJEMPLO 8: purificación general de una proteína de fusión de albúmina expresada a partir de un constructo de fusión de albúmina en líneas celulares mamíferas.

En realizaciones preferidas, las proteína de fusión de albúmina del invento comprenden la forma madura de HSA fusionada ya sea al extremo N- o C- de la forma madura de una proteina terapéutica o porciones de la misma (ej., la forma madura de una proteína terapéutica listada en la Tabla 1, o la forma madura de una proteína terapéutica mostrada en la Tabla 2 como SEQ ID NO:Z). En una realización del invento, las proteínas de fusión de albúmina del invento comprenden además una secuencia de señal la cual dirige el péptido de fusión naciente en las rutas secretorias del huesped utilizado para expresión. En una realización preferida, el péptido de señal codificado por la secuencia de señal es removido, y la proteína de fusión de albúmina madura es secretada directamente en el medio de cultivo. Las proteínas de fusión de albúmina del invento preferiblemente comprenden secuencias de señal heterólogas (ej., la secuencia de señal no nativa de una proteína terapéutica particular) incluyendo, pero sin limitar a, MAF, INV, Ig, Fibulina B, Clusterina, Proteína 4 de Unión de Factor de Crecimiento tipo Insulina, secuencias guía HSA variantes incluyendo, pero sin limitar a una secuencia guía quimérica HSA/MAF, u otras secuencias de señal heterólogas conocidas en el estado de la técnica. Especialmente preferidas son aquellas secuencias de señal listadas en la Tabla 2 y/o la secuencia de señal listada en la sección "Expresión de Proteínas de Fusión" y/o "Métodos Adicionales de Producción Recombinante y Sintética de Proteinas de Fusión de Albúmina" de la especificación anterior. En realizaciones preferidas, las proteínas de fusion del invento además comprenden un residuo de metionina N-terminal. Los polinucleotidos que codifican estos polipéptidos, incluyendo fragmentos y/o variantes, también son contemplados por el invento.

Las proteínas de fusión de albúmina de sobrenadantes de líneas celulares mamíferas son purificadas según diferentes protocolos dependiendo del sistema de expresión utilizado.

Purificación a partir de líneas celulares CHO y 293T.

La purificación de una proteína de fusión de albúmina a partir del sobrenadante de células CHO o partir de sobrenadante de células 293T transfectadas puede involucrar captura inicial con una resina HQ aniónica utilizando amortiguador de fosfato de sodio y una elución de gradiante de fosfato, seguido por cromatografia de afinidad sobre una columna de Sefarosa Blue FF utilizando una elución de gradiente de sal. La Sefarosa Blue FF remueve los principales contaminantes de BSA/fetuina. Purificación adicional sobre la resina Poros PI 50 con un gradiente de fosfato puede remover y reducir contaminación de endotoxina así como también concentrar la proteína de fusión de albúmina.

Purificación a partir de línea celular NS0.

La purificación de una proteína de fusión de albúmina a partir de sobrenadante de células NS0 puede involucrar cromatografia de intercambio aniónico de Q-Sefarosa, seguido por purificación de SP-sefarosa con una elución en etapas, seguida por purificación de Fenil-650M con una elución en etapas, y, finalmente diafiltración.

Luego la proteína purificada puede ser fórmulada mediante intercambio de amortiguador.

EJEMPLO 9: Expresión Bacteriana de una Proteína de Fusión de Albúmina

Un polinucleotidos que codifica una proteína de fusión de albúmina del presente invento comprendiendo una secuencia de señal bacteriana es amplificado utilizando cebadores

oligonucleotidos PCR correspondientes a los extremos 5' y 3' de la secuencia de ADN, para sintetizar fragmentos de insercción. Los cebadores utilizados para amplificar el inserto codificador de polinucleotidos preferiblemente contienen sitios de restricción, tales como BamHI y XbaI, en el extremo 5' de los primeros con el objeto de clonar el producto amplificado en el vector de expresión. Por ejemplo, BamHI y XbaI corresponden a los sitios de restricción enzimática sobre el vector de expresión bacteriano pQE-9. (Qiagen, Inc., Chatsworth, CA). Este vector plásmido codifica resistencia antibiótica (Ampr), un origen bacteriano de replicación (ori), un promotor/regulador regulable por IPTG (P/O), un sitio de unión de ribosoma (RBS), una etiqueta de 6-histidina (6-His), y sitios de clonación de enzimas de restricción.

El vector pQE-9 es digerido con BamHI y XbaI y el fragmento amplificado es ligado en el vector pQE-9 manteniendo el marco de lectura iniciado en RBs bacteriano. Luego la mezcla de ligación es utilizada para transformar la cepa de E. coli M15/rep4 (Qiagen, Inc.) la cual contiene múltiples copias del plásmido pREP4, el cual expresa el represor lac1 y también confiere resistencia a canamicina (Kanr). Los transformantes son identificados por su capacidad para crecer sobre placas LB y las colonias resistentes a ampicilina/canamicina son seleccionadas. El ADN plásmido es aislado y confirmado mediante análisis de restricción.

Los clones que contienen los constructos deseados son desarrollados durante la noche (O/N) en cultivo líquido en medio LB suplementado con Amp (100 ug/ml) y Kan (25 ug(ml). El cultivo O/N se utiliza para inocular un cultivo grande en una proporción de 1:100 a 1:250. Las células son desarrolladas a una densidad óptima 600 (O.D.600) de entre 0.4 y 0.6. Luego IPTG (piranosida de isopropil-B-D-tiogalacto) es agregado a una concentración final de 1 mM. IPTG induce mediante inactivación el represor lacI, clarificando el P/O llevando a una expresión de genes aumentada.

Las células son cultivadas durante 3 a 4 horas más. Luego las células son cosechadas mediante centrifugación (20 min a 6000Xg). La píldora celular es solubilizada en el agente caotrópico HCl de guanidina 6 molar o preferiblemente en urea 8 M y concentraciones mayores que 0.14 M 2-mercaptoetanol mediante agitación durante 3-4 horas a 4°C (ver, eg., Burton, Eur. J. Biochem. *179*:379-378 (1989)). El desecho celular es retirado mediante centrifugación, y el sobrenadante conteniendo el polipéptido es cargado sobre una columna de resina de afinidad de ácido niquel-nitrilo-tri-acético ("Ni-NTA") (disponible en Q1AGEN, Inc., anteriormente). Las proteínas con una etiqueta 6 x His se unen a la resina Ni-NTA con alta afinidad y se pueden purificar en una procedimiento de un paso simple (para detalles ver: The Q1Aexpressionist (1995) QIAGEN, Inc., anteriormente).

Brevemente, el sobrenadante es cargado sobre la columna en 6M guanidina-HCl, pH 8. La columna primero es lavada con 10 volumenes de 6M guanidina-HCl, pH 8, luego lavada con 10 volumenes de 6M guanidina-HCl pH6, y finalmente el polipéptido es eluido con 6 M guanidina-HCl, pH 5.

La proteína purificada luego es renaturalizada al dializar ésta contra solución salina amortiguada por fosfato (PBS) o 50 nM de Na-acetato, amortiguador pH 6 más 200 mM NaCl. Alternativamente, la proteína puede ser exitosamente redoblada mientras es inmovilizada sobre la columna Ni-NTA. Las condiciones de ejemplo son como sigue: renaturalizar utilizando un gradiente de urea 6M-1M en 500 mL de NaCl, glicerol al 20%, 20 mM Tris/HCl pH 7.4, conteniendo inhibidores de proteasa. La renaturalización se debe realizar durante un periodo de 1.5 horas o más. Después de renaturalización las proteínas son eluidas mediante la adición de 250 mM imidazol. El imidazol es retirado mediante un paso final de dialización contra PBS o 50 mM acetato de sodio pH 6 amortiguador más 200 mM NaCl. La proteína purificada es almacenada a 4°C o congelada a -80°C.

Además del vector de expresión anterior, el presente invento además incluye un vector de expresión, llamado pHE4a (Acceso No. ATCC 209645, depositado en Febrero 25 de 1998) el cual contiene elementos de operador y promotor de fagos operativamente ligados a un polinucleotido que codifica una proteína de fusión de albúmina del presente invento, llamado pHE4a. (Acceso ATCC No. 209645, depositado en Febrero 25 de 1998.) Este vector contiene: 1) un gen de neomicinfosfotransferasa como un marcador de selección, 2) un origen de replicación de E. colo, 3) una secuencia promotora de fatos T5, 4) dos secuencias operadoras lac, 5) una secuencia Shine-Delgarno, y 6) el gen represor operador de lactosa (lacIq). El origen de replicación (oriC) es derivado de pUC19 (LTI, Gaithersburg, MD). Las secuencias promotoras y operadoras son hechas de manera sintética.

El ADN se puede insertar en el pHE4a al restringir el vector con Ndel y Xbal, BamHI, Xhol, o Asp718, pasando el producto restringido sobre un gel, y aislando el fragmento mayor (el fragmento más atestado debe ser de aproximadamente 310 pares base). El inserto de ADN es generado según protocolos PCR descritos en la presente o de otra manera conocidos en el estado de la técnica, utilizando cebadores PCR con sitios de restricción para Ndel (cebador 5') y Xbal, BamHI, Xhol, o Asp718 (cebador 3'). El iserto PCR es purificado por gel y restringido con enzimas compatibles. El inserto y vector son ligados según protocolos estándares.

El vector diseñado puede ser substituido en el protocolo anterior para expresar la proteína en un sistema bacteriano.

EJEMPLO 10: Aislamiento de un Clon de cADN Seleccionado a Partir de la Muestra Depositada.

Muchos de los constructos de fusión de albúmina del invento han sido depositados con la ATCC como se muestra en la Tabla 3. Los constructos de fusión de albúmina pueden comprender cualquiera de los siguientes vectores de expresión: el vector de expresión de levadura *S. cerevisiae* pSAC35, el vector de expresión mamífero pC4, o el vector de expresión mamífero pEE12.1.

pSAC35 (Sleep 1990, Biotechnology 8:42), pC4 (Acceso ATCC No. 209646, Cullen. Molecular and Cellular Biology, 438-447 (1985); Boshart, Cell 41: 521-530 (1985)), y pEE12.1 (Lonza Biologics, Inc; Stephens y Cockett, Nucl- Acids Res. 17: 7110 (1989); Publicación Internacional #WO89/01036; Murphy, Biochem J. 227: 277-279 (1991); Bebbington. Bio/Tecnhology 10:169-175 (1992); Patente Americana No. 5.122.464; publicación Internacional #WO86/05807) vectores comprenden un gen de resistencia a ampicilina para crecimiento en células bacterianas. Estos vectores y/o un constructo de fusión de albúmina que los comprende se puede transformar en una cepa de E. coli tal como Stratagene XL-1 Blue (Stratagene Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037) utilizando técnicas descritas en el estado de la técnica tal como Hanahan, diseminados sobre placas de agar Luria-Broth conteniendo 100 uG/mL de ampicilina, y cultivados durante la noche a 37°C.

Al material depositado en la muestra se asignó el Número de Depósito ATCC citado en la Tabla 3 para cualquier constructo de fusión de albúmina dado también puede contener uno o más constructos de fusión de albúmina adicionales, cada uno codificando diferentes proteínas de fusión de albúmina. Asi, los depositos que comparten el mismo Numero de Depósito ATCC contienen al menos un constructo de fusión de albúmina identificado en la correspondiente fila de la Tabla 3.

Se pueden utilizar dos enfoques para aislar un constructo de fusión de albúmina particular a partir de la muestra depositada de ADNs plásmidos citados para ese constructo de fusión de albúmina en la Tabla 3.

Método 1: Clasificación

Primero, un constructo de fusión de albúmina se puede aislar directamente al seleccionar la muestra del ADNs plásmidos depositados utilizando una sonda polinucleotida correspondiente a SEQ ID NO:X para un número ID de constructo individual en la Tabla 1, utilizando métodos conocidos en el estado de la técnica. Por ejemplo, un polinucleotido específico con 30-40 nucleotidos se puede sintetizar utilizando un sintetizador de ADN Applied Biosystems según la secuencia reportada. El oligonucleotido se puede rotular, por ejemplo, con 32P-gama-ATP utilizando quinasa polinucleotida T4 y se puede purificar según métodos de rutina. (Ej., Maniatis Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY (1982)). El constructo de fusión de albúmina de un depósito ATCC dado es transformado en un huesped apropiado, como se indicó anteriormente (tal como XL-1 Blue (Stratagene)) utilizando técnicas conocidas por aquellos con experiencie en el estado de la técnica, tales como aquellas proporcionadas por el proveedor de vectores o en publicaciones relacionadas o patentes citadas anteriormente. Los transformantes son sembrados sobre placas de agar 1.5% (conteniendo el agente de selección apropiado, ej., ampicilina) a una densidad de 150 transformantes aproximadamente (colonias) por placa. Estas placas son seleccionadas utilizando membranas de nailon según métodos comunes para selección de colonias bacterianas (ej., Sambrook, Molecular Cloning: A Laboratory Manual, 2da Edit, (1989), Cold Spring Harbor Laboratory Press, paginas 1.93 a 1.104) u otras técnicas conocidas por aquellos con experiencia en el estado de la técnica.

Método 2: PCR

Alternativamente, el ADN que codifica una proteina de fusión de albúmina dada puede ser amplificado a partir de una muestra de un constructo de fusión de albúmina depositado con SEQ ID NO:X, por ejemplo, al utilizar dos cebadores de 17-20 nucleotidos que hibridizan al constructo de fusión de albúmina depositado 5' y 3' al ADN que codifica una proteína de fusión de albúmina dada. La reacción de cadena de polimerasa es realizada bajo condiciones de rutina, por ejemplo, en 25 ul de mezcla de reacción con 0.5 ug del cADN plantilla anterior. Una mezcla de reacción conveniente es 1.5-5 mM MgCl2, 0.01% (w/v) gelatina, 20 uM cada una de dATP, dCTP, dTTP, 25 pmol de cada cebador y 0.25 Unidades de polimerasa Taq. Treinta y cinco ciclos de PCR (desnaturación a 94°C durante 1 min; endureciendo a 55°C durante 1 minuto; elongación a 72°C durante 1 min) son realizados con un ciclizador térmico automatizado. El producto amplificado es analizado mediante electroforesis de gel de agarosa y la banda de ADN con el peso molecular esperado es cortada y purificada. El producto PCR es verificado para ser la secuencia seleccionada al subclonar y secuenciar el producto de ADN.

Varios métodos están disponibles para la identificación de las porciones no codificadoras 5' y 3' de un gen las cuales pueden no estar presentes en el clon depositado. Estos métodos incluyen pero no están limitados a sondeo de filtro, enriquecimiento de clones utilizando sondas específicas, y protocolos similares o idénticos a protocolos "RACE" 5' y 3' los cuales son conocidos en el estado de la técnica. Por ejemplo, un método similar al 5' RACE es disponible para generar el extremo 5' faltante de un transcripto de longitud total deseado. (Fromont-Racine, Nucleic Acids Res., 21(7):1683-1684 (1983)).

Brevemente, un oligonucleotido de ARN específico está ligado a los extremos 5' de una población de ARN conteniendo presumiblemente transcriptos de ARN de genes de longitud total. Un grupo de cebadores conteniendo un cebador específico al oligonucleotido de ARN ligado y un cebador específico a una secuencia conocida del gen de interés se utiliza para amplificar mediante PCR la porción 5' del gen de longitud total deseado. El producto amplificado luego puede ser secuenciado y utilizado para generar el gen de longitud completa.

Este método anterior arranca con el ARN total aislado a partir de la fuente deseada, aunque se puede utilizar poli-A+ARN. Luego la preparación de ARN se puede tratar con

fosfatasa si es necesario para eliminar grupos de fosfato 5' sobre ARN degradado o dañado el cual puede interferir con el último paso de ligasa de ARN. Luego la fosfatasa debe ser inactivada y el ARN se debe tratar con pirofosfatasa de ácido de tabaco para retirar la estructua de tapa presente en los extremos 5' del ARNs mensajero. Esta reacción deja un grupo de fosfatasa 5' en el extremo 5' del ARN adherido a tapa el cual luego puede ser ligado a un oligonucleotido de ARN utilizando ligasa de T4 ARN.

Esta preparación de ARN modificado se utiliza como un modelo para la primera síntesis de cADN de hebra utilizando un oligonucleotido especifico de gen. La primera reacción de síntesis de hebra se utiliza como un modelo para amplificación PCR del extremo 5' deseado utilizando un cebador específico al oligonucleotido de ARN ligado y un cebador específico a la secuencia conocida del gen de interés. El producto resultante luego es secuenciado y analizado para confirmar que la secuencia final 5' pertenece al gen deseado.

EJEMPLO 11: Fusiones Multifusión

Las proteínas de fusión de albúmina (ej., conteniendo una proteína Terapéutica (o fragmento o variante de la misma) fusionadas a albúmina (o un fragmento o variante de la misma) adicionalmente pueden ser fusionadas a otras proteínas para generar "proteínas multifunción". Estas proteinas multifusión se pueden utilizar para una variedad de aplicaciones. Por ejemplo, fusión de las proteínas de fusión de albúmina del invento a etiqueta-His, etiqueta-HA, proteína A, dominios IgG, y proteína de unión de maltosa facilita la purificación (ver ej., EP A 394.827; Traunecker, Nature 331:84-86 (1988)). Señales de localización nuclear fusionadas a los polipéptidos del presente invento pueden dirigir la proteína a una localización subcelular específica, mientras heterodimeros u homodimeros covalentes pueden aumentar o disminuir la actividad de una proteína de fusión de albúmina. Además, la fusión de secuencias de proteínas adicionales a las proteínas de fusión de albúmina del invento pueden aumentar adicionalmente la solubidad y/o estabilidad de la proteína de fusión. Las proteínas de fusión descritas anteriormente pueden ser hechas utilizando técnicas de modificación comunes conocidas en el estado de la técnica y/o al modificar el siguiente protocolo, el cual describe la fusión de un polipéptido a una molecula IgG.

Brevemente, la porción Fc humana de la molecula IgG puede ser amplificada por PCR, utilizando cebadores que se extienden sobre los extremos 5' y 3' de la secuencia descrita a continuación. Estos cebadores también deben tener sitios de restricción enzimática que facilitarán la clonación en un vector de expresión, preferiblemente un vector de expresión mamífero o de levadura.

Por ejemplo, si se utiliza pC4 (No. de Acceso ATCC 209646), la porción Fc humana puede ser ligada al sitio de clonación BamH. Note que el sitio 3' BamHI debe ser destruido. Luego, el vector que contiene la porción Fc humana es re-restringido con BamHI, linerarizando el vector, y un polinucleotido que codifica una proteína de fusión de albúmina del presente invento (generado y aislado utilizando técnicas conocidas en el estado de la técnica), es ligado a este sitio BamHI. Notar que el polinucleotido que codifica la proteína de fusión del invento es clonado sin un codon de tope, de otra manera no se producirá un Fc que contiene una proteína de fusión.

Si la secuencia de señal se utiliza para producir la proteína de fusión de albúmina del presente invento, pC4 no necesita un segundo péptido de señal. Alternativamente, si la secuencia de señal de ocurrencia natural no es utilizada, el vector se puede modificar para incluir una secuencia de señal heteróloga. (Ver, ej., Publicación Internacional No. WO 96/34891).

Región Fc IgG humana:

GGGATCCGGAGCCCAAATCTTCTGACAAAACTCACACATGCCCACCGTGCCC
AGCACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGA
CACCCTCATGATCTCCCGGACTCCTGAGGTCACATGCGTGGTGGTGGACGTAAGCCA
CGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATG
CCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTC
CTCACCGTCCTGCACCAGGACTGGCTGAATGGAAGGAGTACAAGTGCAAGGTCTCCA
ACAAAGCCCTCCCAACCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCC
CGAGAACCACAGGTGTACACCCTGCCCCATCCCGGATGAGCTGACCAAGAACCAGG
TCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCGCGTGGAGTGGAG
AGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGA
CGGCTCCTTCTTCCTCTACAGCAAGTCACCGTGGACAAGAGCAGGTGGCAGCAGGGG
AACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAG
AGCCTCTCCCTGTCTCCGGGTAAATGAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ
ID NO:52)

EJEMPLO 12: Producción de un anticuerpo a partir de una Proteína de Fusión de Albúmina

Tecnología de Hibridoma

Los anticuerpos que fijan las proteínas de fusión de albúmina del presente invento y porciones de las proteínas de fusión de albúmina del presente invento (ej., la porción de proteína Terapéutica o porción de albúmina de la proteína de fusión) se pueden preparar mediante una variedad de métodos. (ver, Current Protocols, Capitulo 2). Como un ejemplo de tales métodos, una preparación de una proteína de fusión de albúmina del invento o porción de una proteína de fusión de albumina del invento es preparada y purificada para hacerla sustancialmente libre de contaminantes naturales. Luego tal preparación es introducida en un animal para producir antisueros policlonales de mayor actividad específica.

Los anticuerpos monoclonales específicos para una proteína de fusión de albúmina del invento, o una porción de una proteína de fusión de albúmina del invento, son preparados utilizando tecnología de hibridoma (Kohler, Nature 256:495 (1975); Kohler, Eur. J. Immnunol. 6:511 (1976); Kohler, Eur. J. Immnunol. 6:292 (1976); Hammerling, en: Anticuerpos Monoclonales e Hibridomas de Células T, Elsevier, N.Y., pp. 563-681 (1981)). En general, un animal (preferiblemente un ratón) es inmunizado con una proteína de fusión de albúmina del invento, o una porción de una proteína de fusión de albúmina del invento. Los esplenocitos de tales ratones son extraidos y fusionados con una línea células de mieloma apropiado. Cualquier línea de células de mieloma apropiado se puede emplear según el presente invento; sin embargo, es preferible emplear la línea celular de mieloma precursora (SP20), disponible en ATCC. Después de la fusión, las células de hibridoma resultante son selectivamente mantenidas en medio HAT, y luego son clonadas mediante dilución limitante como se describió por Wands (Gastroenterología 80:225-232 (1981)). Las células de hibridoma obtenidas a través de tal selección son luego probadas para identificar clones que secretan anticuerpos capaces de unir una proteína de fusión de albúmina del invento, o una porción de una proteína de fusión de albúmina del invento.

Alternativamente, anticuerpos adicionales capaces de unirse a una proteína de fusión de albúmina del invento, o una porción de una proteína de fusión de albúmina del invento se pueden producir en un procedimiento de dos pasos utilizando anticuerpos anti-idiotípicos. Tal método hace uso del hecho que los anticuerpos son así mismos antígenos, y por lo tanto, es posible obtener un anticuerpo que se una a un segundo anticuerpo. Según este método, los anticuerpos específicos de proteinas se utilizan para inmunizar un animal, preferiblemente un ratón. Luego

los esplenocitos de tal animal son utilizados para producir células de hibridoma, y las células de hibridoma son seleccionadas para identificar clones los cuales producen un anticuerpo cuya capacidad para unirse al anticuerpo específico de una proteína de fusión de albúmina del invento (o porción de una proteína de fusión de albúmina del invento) puede ser bloqueada por la proteína de fusión del invento, o una porción de una proteína de fusión de albúmina del invento. Tales anticuerpos comprenden anticuerpos anti-idiotípicos al anticuerpo específico de la proteína de fusión del invento (o porción de una proteína de fusión de albúmina del invento) y se utilizan para inmunizar un animal para inducir formación de anticuerpos adicionales específicos de proteínas de fusión del invento (o porción de una proteína de fusión de albúmina del invento).

Para uso *in vivo* de anticuerpos en humanos, un anticuerpo es "humanizado". Tales anticuerpos se pueden producir utilizando constructos derivados de células de hibridoma produciendo los anticuerpos monoclonales descritos anteriormente. Los métodos para producir anticuerpos quiméricos y humanizados son conocidos en el estado de la técnica y son discutidos en la presente. (Ver, Morrison, Science 229:1202 (1985); Oi, BioTechniques 4:214 (1986); Cabilly Patente Americana No. 4.816.567; Taniguchi, EP 171496; Morrison, EP 171496, Morrison, EP 173494; Neuberger, WO 8601533; Robinson. Publicación Internacional No. WO 8702671; Boulianne, Nature 312:643 (1984); Neuberger, Nature 314:268 (1985)).

Aislamiento de Fragmentos de Anticuerpos Dirigidos Contra una proteína de fusión de albúmina del invento, o una porción de una proteína de fusión de albúmina del invento a partir de una Biblioteca de scFvs. Genes-V de ocurrencia natural aislados a partir de PBLs humanos son construidos en una biblioteca de fragmentos de anticuerpos los cuales contienen reactividades contra una proteína de fusión de albúmina del invento, o una porción de una proteína de fusión de albúmina del invento, a los cuales el donante puede o no haber sido expuesto (ver ej., Patente Americana No. 5.885.793 incorporada en la presente como referencia en su totalidad).

Recuperación de la Biblioteca. Una biblioteca de scFVs es construida a partir del ARN de PBLs humanos como se describió en la Publicación Internacional No. WO 92/01047. Para recuperar fragmentos de anticuerpos de presentación de fagos, aproximadamente 10 a la 9 *E. coli* albergando el fagomid se utilizan para inocular 50 ml de 2xTY conteniendo glucosa al 1% y 100 ug/ml de ampicilina (2xTY-AMP-GLU) y desarrollada a un O.D. de 0.8 con agitación. Cinco ml de este cultivo se utilizan para inocular 50 ml de 2xTY-AMP-GLU, 2 x 108 TU de gen delta 3 auxiliar (M13 delta gen III, ver Publicación Internacional No. WO92/01047 son agregados y el cultivo es inoculado a 37°C durante 45 minutos sin agitación y luego a 37°C durante 45 minutos con agitación. El cultivo es centrifugado a 4000 rpm durante 10 minutos y el pellet es resuspendido en 2 litros de 2xTY conteniendo 100 ug/ml de ampicilina y 50 ug/ml de canamicina y desarrollado durante la noche. Los fagos son preparados como se describió en la Publicación Internacional No. WO 92/01047.

El gen delta M13 es preparado como sigue: El fago auxiliar del gen delta M13 no codifica la proteína de gen III, por lo tanto los fragmentos de anticuerpos de presentación de fagos (mid) tienen mayor fuerza de unión a antígeno. Las particulas infecciosas del gen III delta M13 son hechas al cultivar fagos auxiliares en células que albergan un derivado de pUC19 proporcionando la proteína del gen III tipo silvestre durante morfogenesis de fagos. El cultivo es incubado durante 1 hora a 37°C sin agitación y luego durante 1 hora adicional a 37°C con agitación. Las células son spun down (IEC-Centra 8400 rpm durante 10 min), resuspendidas en 300 ml de caldo de 2xTY conteniendo 100 ug de ampicilina/ml y 25 ug de canamicina/ml (2xTY-AMP-KAN) y desarrolladas durante la noche, agitando a 37°C. Las particulas de fagos son purificadas y concentradas a partir de medio de cultivo mediante dos precipitaciones-PEG (Sambrook, 1990), resuspendidas en 2 ml PBS y pasadas a través de un filtro de 0.45 um. (Minisart NML; Sartorius)

para proporcionar una concentración final de aproximadamente 10 a la 13 unidades de transducción/ml (clones resistentes de ampicilina).

Toma Panorámica de la Biblioteca. Imunotubos (Nunc) son recubiertos durante la noche en PBS con 4 ml de 100 ug/ml o 10 ug/ml de una proteína de fusión de albúmina del invento, o una porción de una proteína de fusión de albúmina del invento. Los tubos son bloqueados con PBS-Marvel al 2% durante 2 horas a 37°C y luego lavados 3 veces en PBS. Aproximadamente 10 a la 13 TU de fagos es aplicado al tubo e incubado durante 30 minutos a temperatura ambiente tumbling on an over and underturntable y luego dejando sentar durante otras 1.5 horas. Los tubos son lavados 10 veces con PBS 0.1% Tween-20 y 10 veces con PBS. Los fagos son eluidos agregando 1 ml de 100 mM trietilamina y rotando 15 minutos sobre un under and over turntable después de lo cual la solución es inmediatamente neutralizada con 0.5 ml de 1.0 M Tris-HCl, pH 7.4. Luego los fagos se utilizan para infectar 10 ml de mid-log E. coli TG1 incubando los fagos eluidos con bacterias durante 30 minutos a 37°C. Luego E. coli se siembra en placas sobre placas TYE conteniendo glucosa al 1% y 100 ug/ml de ampicilina. Luego la biblioteca bacteriana resultante es recuperada con fagos auxiliares de gen delta 3 como se describió anteriormente para preparar fagos para una ronda subsecuente de selección. Luego este proceso se repite para un total de 4 rondas de purificación de afinidad con lavado de tubos aumentado a 20 veces con PBS, 0.1% Tween-20 y 20 veces con PBS para rondas 3 y 4.

Caracterización de Aglomerantes. Los fagos eluidos de la tercera y cuarta ronda de selección son utilizados para infectar E. coli HB 2151 y scFv soluble es producido (Marks, 1991) a partir de colonias simples para ensayo. Se realizan ELISAS con placas de microtitulos recubiertas con 10 pg/ml de una proteína de fusión de albúmina del invento, o una porción de una proteína de fusión de albúmina del invento, en 50 mM de bicarbonato pH 9.6. Clones positivos en ELISA son caracterizados adicionalmente mediante toma de huellas PCR (ver, ej., Publicación Internacional No.g WO 92/01047) y luego por secuenciamiento. Estos clones ELISA-positivos también se pueden caracterizar adicionalmente mediante técnicas conocidas en el estado de la técnica, tal como, por ejemplo, mapeo de epítopes, afinidad de unión, transducción de señales de receptor, capacidad par bloquear o inhibir competitivamente unión de anticuerpos/antígenos, y actividad agonista o antagonista competitiva.

EJEMPLO 13: Ensayo de Absorción de [3H]-2-Desoxiglucosa:

Los tejidos adiposo, musculo esqueletico, y hepático son tejidos sensibles a insulina. La insulina puede estimular la absorción/trasporte de glucosa hacia estos tejidos. En el caso de tejido adiposo y musculo-esquelético, la insulina inicia la transducción de señales que eventualmente lleva a la translocación de la molécula transportadora de glucosa 4, GLUT4, desde un compartimiento intracelular especializado hacia la superficie celular. Una vez en la superficie celular, GLUT4 permite la absorción/trasporte de glucosa.

Absorción de [3H]-2-Desoxiglucosa

Un número de líneas celulares relacionadas con tejido adiposo y muscular se pueden utilizar para probar la actividad de absorción/transporte de glucosa en ausencia o presencia de una combinación de uno o más fármacos terapéuticos listados para el tratamiento de diabetes mellitus. En particular, las células de fibroblastos murinos de 3T3-L1 y las células musculo esqueleticas murinas L6 se pueden diferenciar en adipocitos 3T3-L1 y en mitubos, respectivamente, para servir en modelos *in vitro* para el ensayo de absorción de [3H]-2-desoxiglucosa (Urso, J. Biol Chem, 274(43): 30864-73 (1999); Wang, J. Mol Endocrinol, 19(3): 241-8 (1991); Haspel, J Membr Biol, 169 (1): 45:53 (1999); Tsakiridis, Endocrinology, 136(10): 4315-22 (1995)). Brevemente, 2 x 10 5 células/100 uL de adipocitos o células L6 diferenciadas son transferidas a Cultivo de Tejido de 96 pozos, "TC", tratado, es decir, recubierto con 50

ug/mL de poli-L-lisina, placas en medio de post-diferenciación y son incubadas durante la noche a 37°C en CO2 al 5%. Primero las células son lavadas una vez con medio DMEM de glucosa bajo libre de suero y luego son despojadas con 100 uL/pozo del mismo medio con 100 uL/pozo de ya sea amortiguador o de una combinación de uno o más de los fármacos terapéuticos listados para el tratamiento de diabetes mellitus, por ejemplo, concentraciones crecientes de 1 nM, 10 nM, y 100 nM de los terapeuticos del invento (ej., fusiones específicas divulgadas como SEQ ID NO:Y y fragmentos y variantes de la misma) durante 16 horas a 37°C en ausencia o presencia de 1 nM insulina. Las placas son lavadas tres veces con 100 uL/pozo de solución salina amortiguada con HEPES. Se agrega insulina a 1 nM en solución salina amortiguada por HEPES durante 30 minutos a 37°C en presencia de 10 uM [3H]-2-desoxiglucosa (Amersham, #TRK672) yg 10 uM de 2-desoxiglucosa no rotulada (SIGMA, D-3179). Como control, las mismas condiciones son realizadas excepto en la ausencia de insulina. Una concentración final de 10 uM citocalasina B (SIGMA, C6762) es agregada a 100 uL/pozo en una pozo separado para medir la absorción no específica. Las células son lavadas tres veces son solución salina amortiguada con HEPES. Rotulada, es decir, 10 uM de [3H]-desoxiglucosa, y no rotulada, es decir, 10 uM de 2-desoxiglucosa, se agregan durante 10 minutos a temperatura ambiente. Las células son lavadas tres veces con Solución Salina Amortiguada con Fosfato frio, "PBS". Las células son sometidas a lisis ante la adición de 150 uL/pozo de 0.2 N NaOH e incubación subsiguiente con agitación durante 20 minutos a temperatura ambiente. Luego las muestras son transferidas a un recipiente de centelleo al cual se agregó 5 mL de fluido de centelleo. Los recipientes son contados en un contador Beta-Centelleo. Absorción en condiciones duplicadas, la diferencia como la ausencia o presencia de insulina, es determinada con la siguiente ecuación: [(totales de insulina por minuto "cpm" - cpm no específico)/(cpm no insulina - cpm no específico)]. Las respuestas promedio caen dentro de los límites de 5 veces y 3 veces aproximadamente en comparación con los controles para adipocitos y miotubos, respectivamente.

Diferenciación de Células

Las células son dejadas volverse completamente confluentes en un frasco de T-75 cm2. El medio es retirado y reemplazado con 25 mL de medio de pre-diferenciación durante 48 horas. Las células son incubadas a 37°C, en CO2 al 5%, humedad 85%. Después de 48 horas, el medio de pre-diferenciación es retirado y reemplazado con 25 mL de medio de diferenciación durante 48 horas. Las células son nuevamente incubadas a 37°C, en CO2 al 5%, 85 humedad. Después de 48 horas, el medio es retirado y reemplazado con 30 mL de medio de post-diferenciación. El medio de post-diferenciación es mantenido durante 14-20 días o hasta lograr diferenciación completa. El medio es cambiado cada 2-3 días. Los adipocitos humanos se pueden comprar en Zen-Bio. INC (#SA-1096).

EJEMPLO 14: Ensayo in vitro de Incorporación de [3H]-Timidina en Líneas Celulares Pancreaticas.

Recientemente se ha mostrado que GLP-1 induce diferenciación de la línea celular epitelial ductal pancreática de rata ARIP en una forma dependiente de tiempo y dosis la cual está asociada con un aumento en niveles de Homebox-1-Duodenal de Isletas (IDX-1) y mARN de insulina (Hui, 2001, Diabetes, 50(4): 785-96). El IDX-1 a su vez aumenta los niveles de mARN del receptor GLP.

Tipos Celulares Probados

Células RIN-M: Estas células están disponibles en American Type Tissue Culture Collection (Número de Línea Celular ATCC CRL-2057). La línea celular RIN-M fue derivada de un tumor de células de isletas de rata trasplantables inducidas por radiación. La línea fue establecida a partir de xenoinjerto de ratón desnudo del tumor. Las células producen y secretan

hormonas polipeptídicas de isletas, y producen descarboxilasa de L-dopa (un marcador para células que tienen captación y descarboxilación de precursor de amina, o actividad APUD).

Células ARIP: Estas son células exocrinas pancreaticas de morfología epitelial disponible en American Type Tissue Culture Collection (Número de Línea Celular ATCC CRL-1674). Ver también, referencias: Jessop, N.W. y Hay, R.J. "Características de dos líneas celulares exocrinas pancreaticas de rata derivadas de tumores transplantables", in Vitro 16: 212, (1980); Cockell, "identificación de una actividad de unión de ADN específica a células que interactua con un activador transcripcional de genes expresado en el pancreas acinar," Mol. Cell. Biol. 9: 2464-2476, (1989); Roux, E. "El factor de transcripción específico a células PTF1 contiene dos subunidades diferentes que interactuan con el ADN" Genes Dev. 3: 1613-1624, (1989); y Hui, H. "Peptido 1 tipo glucagon induce diferenciación de células ductales pancreaticas homebox-1-positivas duodenales de isletas en células secretoras de insulina," Diabetes 50: 785-796 (2001).

Preparación de Células

La línea celular RIN-M es desarrollada en medio RPMI 1640 (hyclone, #SH300027.01) con suero bovino fetal al 10% (hyClone, #SH30088.03) y es subcultivada cada 6 a 8 días a una proporción de 1:3 a 1:6. El medio es cambiado cada 3 a 4 días.

La línea celular ARIP (ATCC #CRL-1674) es desarrollada en medio F12K de Ham (ATCC, #30-2004) con 2mM L-glutamina ajustada para contener 1.5 g/L de bicarbonato de calcio y suero bovino fetal al 10%. La línea celular ARIP es subcultivada a una proporción de 1:3 a 1:6 dos veces por semana. El medio es cambiado cada 3 a 4 días.

Protocolo de Ensayo

Las células son sembradas a 4000 células/pozo en placas de 96 pozos y cultivadas durante 48 a 72 horas a confluencia de 50%. Las células son cambiadas a medio libre de suero a 100 uL/pozo. Después de incubación durante 48-72 horas, el suero y/o los terapeuticos del invento (ej., proteínas de fusión de albúmina del invento y fragmentos y variantes de los mismos) son agregados al pozo. La incubación persiste durante 36 horas más. [3H]-Timidina (5-20 Ci/mmol) (Amersham Pharmacia, #TRK120) es diluido a 1 microCuries/5 microtítulos. Después de 36 horas de incubación, se agregan 5 microtítulos por pozo durante 24 horas más. La reacción termina al lavar las células suavemente con sustancia salina fria amortiguada con fosfato, "PBS", una vez. Luego las células son fijadas con 100 microtítulos de TCA helado al 10% durante 15 min a 4°C. El PBS es retirado y se agregan 200 microtítulos de 0.2 N NaOH. Las placas son incubadas durante 1 hora a temperatura ambiente con agitación. La solución es transferida a un recipiente de centelleo y 5 mL de fluido de centelleo compatible con soluciones acuosas es agregado y mezclado vigorosamente. Los recipientes son contados en un contador de beta-centelleo. Como control negativo, solamente se utilizó amortiguador. Como control positivo se utilizó suero de ternero fetal.

EJEMPLO 15: Probando para Glicosuería

Glicosuria (es decir, azucar de exceso en la orina), se puede probar facilmente para proporcionar un índice del estado patológico de diabetes mellitus. La orina de exceso en una muestra de paciente en comparación con una muestra de paciente normal es sintomática de IDDM y NIDDM. La eficacia de tratamiento de tal paciente con IDDM y NIDDM es indicada mediante una disminución resultante en la cantidad de glucosa de exceso en la orina. En una realización preferida para monitoreo de IDDM y NIDDM, se prueban muestras de orina de pacientes para la presencia de glucosa utilizando técnicas conocidas en el estado de la técnica. La glicosuria en humanos se define por una concentración de glucosa urinaria que excede 100 mg por 100 mL. Los niveles de azucar de exceso en aquellos pacientes que exhiben glicosuuria se

pueden medir de manera aún más precisa al obtener muestras de sangre y probar la glucosa de suero.

EJEMPLO 16: Ensayos que Detectan Estimulación o Inhibición de Diferenciación y Proliferación de Células B.

La generación de respuesas inmunes humorales funcionales requiere señalización soluble y cognada entre células de linaje B y su microambiente. Las señales pueden impartir un estimulo positivo que permite a una célula de linaje nB continuar su desarrollo programado, o un estimulo negativo que instruye a la célula para detener su ruta de desarrollo actual. Hasta ahora, numerosas señales estimuladoras e inhibitorias han sido encontradas por influenciar sensibilidad de células B incluyendo IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-13, IL-14 y Il-15. De manera interesante, estas señales son por si mismas efectores debiles pero pueden, en combinación con varias proteínas co-estimulatorias, inducir activación, proliferación, diferenciación, retorno, tolerancia y muerte entre poblaciones de células B.

Una de las mejores clases estudiadas de proteínas co-estimulatorias de células B es la superfamilia TNF. Dentro de esta familia CD40, CD27, y CD30 junto con sus respectivos ligandos CD154, CD70, y CD153 han sido encontrados regulan una variedad de respuestas inmunes. Los ensayos que permiten la detección y/o observación de la proliferación y diferenciación de estas poblaciones de células B y sus precursores son herramientas valiosas para determinar los efectos que varias proteínas pueden tener sobre estas poblaciones de células B en términos de proliferación y diferenciación. Listados a continuación se encuentran dos ensayos diseñados para permitir la detección de la diferenciación, proliferación, o inhibición de poblaciones de células B y sus precursores.

En ensayo in vitro- las proteínas de fusión de albúmina del invento (incluyendo proteínas de fusión que contienen fragmentos o variantes de proteínas Terapeuticas y/o albúmina o fragmentos o variantes de albúmina) se pueden evaluar en cuanto a su capacidad para inducir activación, proliferación, diferenciación o inhibición y/o muerte en poblaciones de células B y sus precursores. La actividad de una proteína de fusión de albúmina del invento sobre células B tonsilares humanas purificadas, medida cualitativamente sobre el rango de dosis entre 0.1 y 10.000 ng/mL, es evaluada en un ensayo de co-estimulación de linfocitos -B estándar en el cual las células B tonsilares purificadas son cultivadas en presencia de *Staphylococus aurens* fijado a formalina Cowan I (SAC) o anticuerpo IgM antihumano inmovilizado como el agente cebador. Segundas señales tales como IL-2 y IL-15 sinergizan con SAC y entrecruzamiento IgM para producir proliferación de células B como se midió mediante incorporación de timidina triada. Agentes sinergizantes novedosos se pueden identificar facilmente utilizando este ensayo. El ensayo involucra aislamiento de células B tonsilares humanas mediante agotamiento de perlas magnéticas (MAC) de células CD3-positivas. La población de células resultantes es mayor que el 95% de células B evaluadas mediante expresión de CD45R(B220).

Varias diluciones de cada muestra son colocadas en pozos individuales de una placa de 96 pozos a la cual se agregan 10 5 células B suspendidas en medio de cultivo (RPMI 1640 conteniendo FBS al 10%, 5 x 10-5 M 2ME, 100U/ml penicilina, 10 ug/ml estreptomicina, y 10-5 dilución de SAC) en un volumen total de 150 ul. La proliferación o inhibición es cuantificada mediante un pulso de 20h (LuCi/pozo) con 3H-timidina (6.7 Vi/mM) comenzando 72h después de adición de fator. Los controles positivos y negativos son IL2 y medio respectivamente.

Ensayo in vivo - Ratones BALB/c son inyectados (ip) dos veces por día con amortiguador solamente, o 2 mg/Kg de una proteína de fusión de albúmina del invento (incluyendo proteínas de fusión conteniendo fragmentos o variantes de proteínas Terapéuticas y/o albúmina o fragmentos o variantes de albúmina). Los ratones reciben este tratatamiento durante 4 días

consecutivos, después de lo cual son sacrificados y varios tejidos y suero son recolectados para análisis. La comparación de secciones de H&E de bazos normales y bazos tratados con la proteína de fusión de albúmina del invento identifica los resultados de la actividad de la proteína de fusión sobre células de bazo, tal como la difusión de envolturas linfáticas peri-arteriales, y/o aumentos significativos en la celularidad nucleada de las regiones de pulpa roja, las cuales pueden indicar la activación de la diferenciación y proliferación de poblaciones de células B. Estudios inmunohistoquímicos que utilizan un marcador de células B, anti-CD45R(B220), se utilizan para determinar si cualquier cambio fisiológico a células esplénicas, tal como desorganización esplénica, se debe a representación aumentada de células B dentro de zonas de células B libremente definidas que infiltran regiones de células T establecidas.

Análisis citométrico de flujo de los bazos de ratones tratados con la proteína de fusión de albúmina se utiliza para indicar si la proteína de fusión de albúmina especificamente aumenta la proporción de ThB+, células CD45R(B220)dull B sobre aquella observada en ratones de control.

Igualmente, una consecuencia pronosticada de representación aumentada de células B maduras *in vivo* es un aumento relativo en titulos de suero Ig. Por consiguiente, los niveles IgM y IgA de suero son comparados entre ratones tratados de amortiguador y proteína de fusión.

EJEMPLO 17: Ensayo de Proliferación de Células T

Un ensayo de proliferación inducido por CD-3 es realizado sobre PBMCs y es medido por la absorción de 3H-timidina. El ensayo es realizado como sigue. Placas de 96 pozos son recubiertas con 100 ul/pozo de mAb a CD3 (HIT3a, Pharmingen) o control mAb (B33.1) durante la noche a 4 grados C (1 ug/ml en .05M amortiguador de bicarbonato, pH 9.5), luego se lavó tres veces con PBS. PBMC son aislados mediante centrifugación de gradiente F/H a partir de sangre humana periférica y se agregó a pozos cuadriplicados (5 x 104/pozo) de placas recubiertas con mAb en RPMI conteniendo FCS al 10% y P/S en presencia de concentraciones variables de una proteína de fusión de albúmina del invento (incluyendo proteínas de fusión que contienen fragmentos o variantes de proteínas Terapéuticas y/o albúmina o fragmentos o variantes de albúmina) (volumen total 200 ul). Amortiguador de proteína relevante y medio solos son controles. Después de 48 horas de cultivo a 37°C, las placas son giradas durante 2 min a 1000 rpm y 100 ul de sobrenadante es removido y almacenado a -20 grados C para medición de IL-2 (u otras citoquinas) si se observa efecto sobre proliferación. Los pozos son bien suplementados con 100 ul de medio conteniendo 0.5 uCi de 3H-timidina y cultivado a 37 grados c durante 18-24 horas. Los pozos son cosechados y la incorporación de 3H-timidina se utilizó como una medida de proliferación. Anti-CD3 solo es el control positivo para proliferación. IL-2 (100 U/ml) también se utilizó como un control el cual aumenta la proliferación. El anticuerpo de control que no induce proliferación de células T se utiliza como el control negativo para los efectos de proteínas de fusión del invento.

EJEMPLO 18: Efecto de Proteínas de Fusión del Invento sobre la Expresión de MHC Clase II, Moléculas Coestimulatoras y de Ahesión y Diferenciación Celular de Monocitos y Células Dendriticas Humanas Derivadas de Monocitos.

Las células dendríticas son generadas por la expansión de precursores de proliferación encontrados en la sangre periférica: PBMC adherente o fracciones monocíticas elutriadas son cultivadas durante 7-10 días con GM-CSF (50 ng/ml) e IL-4 (20 ng/ml). Estas células dendríticas tienen el fenotipo característico de células inmaduras (expresión de CD1, CD80, CD86, CD40 y antígenos MHC clase II). Tratamiento con factores activadores, tales como TNF-alfa, produce un cambio rápido en fenotipo de superficie (expresión aumentada de MHC clase I y II,g moléculas constimulatorias y de adhesión, regulación negativa de FCgamaRII, regulación positiva de

CD83). Estos cambios se correlacionan con capacidad aumentada de presentación de antígenos y con maduración funcional de las células dendriticas.

Análisis FACS de antígenos de superficie es realizado como sigue. Las células son tratadas 1-3 días con concentraciones crecientes de una proteína de fusión de albúmina del invento o LPS (control positivo), lavadas con PBS conteniendo 1% BSA y 0.02 mM azida de sodio, y luego incubadas con 1:20 dilución de FITC apropiado o anticuerpos monoclonales PE rotulados durante 30 minutos a 4 grados C. Después de un lavado adicional, las células rotuladas son analizadas mediante citrometria de flujo sobre un FACScan (Becton Dickinson).

Efecto sobre la producción de citoquinas. Las citoquinas generadas por células dendríticas, en particular IL-12, son importantes en la iniciación de respuestas inmunes dependientes de células-T. IL-12 influye fuertemente sobre el desarrollo de respuesta inmune de células T auxiliares Th1, e induce función celular T y NK citotóxica. Se utiliza un ELISA para medir la liberación de IL-12 como sigue. Las células dendríticas (10 6/ml) son tratadas con concentraciones crecientes de una proteína de fusión de albúmina del invento durante 24 horas. Se agregó LPS (100 ng/ml) al cultivo celular como control positivo. Los sobrenadantes del cultivo celular luego son recolectados y analizados para contenido de IL-12 utilizando un kit ELISA comercial (ej., R & D Systems (Minneapolis, MN)). Los protocoloes estándares proporcionados con los kits son utilizados.

Efecto sobre la expresión de MHC Clase II, moléculas coestimuladoras y de adhesión. Tres familias principales de antígenos de superficie celular pueden ser identificados sobre monocitos: moléculas de adhesión, moléculas involucradas en presentación de antígenos, y receptor Fc. La modulación de la expresión de antígenos MHC clase II y otras moleculas coestimuladoras, tales como B7 y ICAM-1, puede resultar en cambios en la capacidad presentadora de antígenos de monocitos y capacidad para inducir activación de células T. La expresión aumentada de receptores Fc puede correlacionar con actividad citotóxica mejorada de monocitos, liberación de citoquinas y fagocitosis.

El análisis FACS se utiliza para examinar los antígenos de superficie como sigue. Los monocitos son tratados 1-5 días con concentracions crecientes de una proteína de fusión de albúmina del invento o LPS (control positivo), lavados con PBS conteniendo BSA al 1% y 0.02 mM de azida de sodio, y luego incubados con dilución 1:20 de anticuerpos monoclonales PITC o PE monoclonales durante 30 minutos a 4 grados C. Después de una lavada adicional, las células rotuladas son analizadas mediante citometria de flujo sobre un FACScan (Becton Dickinson).

Activación de monocitos y/o superviviencia aumentada. Los ensayos para moleculas que activan (o alternativamente, inactivan) monocitos y/o aumentan supervivencia de monocitos (o alternativamente, disminuyen superviviencia de monocitos) son conocidos en el estado de la técnica y se pueden aplicar de manera rutinaria para determinar si una molécula del invento funciona como un inhibidor o activador de monocitos. Las proteínas de fusión del albúmina del invento se pueden clasificar al utilizar los tres ensayos descritos a continuación. Para cada uno de los tres ensayos, células mononucleares de sangre periférica (PBMC) son purificadas a partir de leucopaquetes de donantes simples (American Red Cross. Baltimore, MD) mediante centrifugación a través de un gradiente de Histopaque (Sigma). Los monocitos son aislados a partir de PBMC mediante elutriación centrífuga de contraflujo.

Ensayo de Supervivencia de Monocitos. Los monocitos de sangre periférica humana progresivamente pierden viabilidad cuando se cultivan en ausencia de suero u otros estimulos. Su muerte resulta de procesos regulados de manera interna (apoptosis). Además del cultivo de factores activadores, tal como TNF-alfa mejora dramaticamente la supervivencia de células y evita la fragmentación de ADN. Se utiliza teñido de yoduro de propidio (PI) para medir apoptosis

como sigue. Los monocitos son cultivados durante 48 horas en tubos de polipropileno en medio libre de suero (control positivo), en presencia de 100 ng/ml TNF-alfa (control negativo), y en la presencia de concentraciones variables de la proteína de fusión a ser probada. Las células son suspendidas a una concentración de 2 x 10 6/ml en PBS conteniendo PI a una concentración final de 5 ug/ml, y luego incubadas a temperatura ambiente durante 5 minutos antes de análisis FACScan. Se ha demostrado que la absorción de PI esta relacionada con la fragmentación de ADN en este paradigma experimental.

Efecto sobre liberación de citoquinas. Una función importante de monocitos/macrofagos es su actividad reguladora sobre otras poblaciones celulares del sistema inmune a través de la liberación de citoquinas después de estimulación. Un ELISA para medir la liberación de citoquinas es realizado como sigue. Los monocitos humanos son incubados a una densidad de 5x105 células/ml con concentraciones crecientes de una proteína de fusión de albúmina del invento y bajo las mismas condiciones, pero en ausencia de la proteína de fusión. Para producción de IL-12, las células son cebadas durante la noche con IFN (100 U/ml) en presencia de la proteína de fusión. Luego se agregá LPS (10 ng/ml). Medios acondicionados son recolectados después de 24 horas y mantenidos congelados hasta su uso. Luego la medición de TNF-alfa, IL-10, MCP-1 y IL-8 es realizada utilizando un kit ELISA comercialmente disponible (ej., R & D Systems (Minneapolis, MN) y aplicando los protocolos estándares proporcionados con el kit.

Incremento Oxidativo. Los monocitos purificados son sembrados en una placa de 96 pozos a 2-1x105 células/pozo. Concentraciones crecientes de una proteína de fusión de albúmina son agregadas a los pozos en un volumen total de 0.2 ml de medio de cultivo (RPMI 1640 + 10% FCS, glutamina y antibioticos). Después de 3 días de incubación, las placas son centrifugadas y el medio es removido de los pozos. A las monocapas de macrofagos, se agregó 0.2 ml por pozo de solución de rojo de fenol (140 mM NaCl, 10 mM de amortiguador de fosfato de potasio pH 7.0, 5.5 mM dextrosa, 0.56 mM rojo de fenol y y 10 U/ml de HRPO), junto con el estimulante (200 nM PMA). Las placas son incubadas a 37°C durante 2 horas y la reacción es detenida al agregar 20 ul 1N NaOH por pozo. La absorbancia es leida a 610 nm. Para calcular la cantidad de H2O2 producido por los macrofagos, una curva estándar de una solución H2O2 de molaridad conocida es realizada para cada experimento.

EJEMPLO 19: El Efecto de Proteínas de Fusión de Albúmina del Invento sobre el Crecimiento de Células Endoteliales Vasculares.

El día 1, las células endoteliales de vena umbilical humana (HUVEC) son sembradas a 2-5x104 células/35 mm de densidad de plato en medio M199 conteniendo suero bovino fetal al 4% (FBS), 16 unidades/ml heparina, 50 unidades/ml de suplementos de crecimiento celular endotelial (ECGS, Biotechnique, Inc.). El día 2, el medio es reemplazado con M199 conteniendo FBS al 10%, 8 unidades/ml de heparina. Una proteína de fusión de albúmina del invento, y controles positivos, tal como VEGF y FGF (bFGF) son agregados, a concentraciones variables. En los días 4 y 6, el medio es reemplazado. El día 8, el numero de células es determinado con un contador Coulter.

Un aumento en el número de células HUVEC indica que la proteína de fusión puede proliferar células vasculares endoteliales, mientras una disminución en el número de células HUVEC indica que la proteína de fusión inhibe células endoteliales vasculares.

EJEMPLO 20: Modelo de Curación de Herida Corneal de Rata.

Este modelo animal muestra el efecto de una proteína de fusión de albúmina del invento sobre neovascularización. El protocolo experimental incluye:

Hacer una incisión de 1-1.5 mm de longitud desde el centro de la cornea hacia la capa estromal.

Insertar una espatula por debajo del borde de la incisión enfrentado la esquina exterior del ojo.

Hacer una bolsa (su base es 1-1.5 mm desde el borde del ojo).

Colocar una píldora, conteniendo 50ng- 5 ug de una proteína de fusión de albúmina del invento, dentro de la bolsa.

El tratamiento con una proteína de fusión de albúmina del invento también se puede aplicar de manera tópica a las heridas corneales en un rango de dosificación de 20mg - 500 mg (tratamiento diario durante cinco días).

EJEPLO 21: Modelos de Curación de Heridas de Ratón Diabético y Deteriorada por Glucocorticoides.

Modelo de Ratón Diabético db+/db+.

Para demostrar que un proteína de fusión de albúmina del invento acelera el proceso de curación, se utiliza el modelo de ratón geneticamente diabético de curación de heridas. El modelo de curación de heridas de espesor completo en ratón db+/db+ es un modelo bien caracterizado, clinacamente relevante y reproducible de curación deteriorada de heridas. La curación de la herida diabética es dependiente de la formación de tejido de granulación y re-epitilialización más que contracción (Gartner, M.H. *J. Surg. Res. 52:*389 (1992); Greenhalgh, D.G. *Am. J. Pathol. 136:* 1235 (1990)).

Los animales diabeticos tienen muchos aspectos característicos observados en diabetes mellitus Tipo II. Los ratones homocigosos (db+/db+) son obesos en comparaciones con sus compañeros de camada heterocigosos normales (db+/+m). Los ratones diabéticos mutantes (db+/db+)tienen una mutación recesiva autosomal simple en el cromosoma 4 (db+) (Colmeman *Proc. Natl. Sci USA 77:*283-293 (1982)). Los animales muestran polifagia, polidipsia, poluria. Los ratones diabéticos mutantes (db+/db+) tienen glucosa sanguinea elevada, niveles aumentados o normales de insulina, e inmunidad suprimida mediada por células (Mandel *J. Immnunol. 120:*1375 (1978); Debray-Sachs, M. *Clin. Exp. Immunol. 51(1):*1-7 (1983); Leiter *Am J. of Pathol. 114:*46-55 (1985)). Neuropatia periférica, complicaciones miocardiales, y lesiones microvasculares, engrosamiento de membrana basal y anomalias de filtración glomerular se han descrito en estos animales (Norido, F. *Exp. Neurol. 83(2):*221-232 (1984); Robertson, *Diabetes 29(1):*60-67 (1980); Giacomelli *Lab Invest. 40(4)*: 460-473 (1979); Coleman, D. L. *Diabetes* 31 (*Suppl*):1-6 (1982)). Estos ratones diabéticos homocigosos desarrollan hiperglicemia que es resistente a análogo de insulina a diabetes humana tipo II (Mandel *J. Immunol. 120:*1375-1377 (1978)).

Las características observadas en estos animales indican que la curación en este modelo puede ser similar a la curación observada en diabetes humana (Greenhalgh, *Am. J. of Pathol.* 136:1235-1246 (1990)).

Ratones hembra diabéticos C57BL/KsJ (db+/db+) y sus compañeros de camada heterocigosos no diabéticos (db+/+m) se utilizaron en este estudio (Jackson Laboratories). Los animales son comprados a las 6 semanas de edad y tienen 8 semanas de edad al comienzo del estudio. Los animales son individualmente albergados y recibieron alimento y agua sin restricción. Todas las manipulaciones son realizadas utilizando técnicas asépticas. Los experimentos son realizados según las normas y pautas de Human Genome Sciences, Inc. El Comite Institucional de Atención y Uso Animal y las Pautas para el Cuidado y Utilización de Animales de Laboratorio.

El protocolo de herida es realizado según métodos anteriormente reportados (Tsuboi, R. Rifkin, D.B., *J. Exp. Med. 172*:245-251 (1990)). Brevemente, el día de la realización de la herida, los animales son anestesiados con una inyección intraperitoneal de Avertin (0.01 mg/mL), 2,2,2-tribromoetanol y 2-metil-2-butanol disuelto en agua desionizada. La región dorsal de animal es afeitada y la piel es lavada con solución de etanol al 70% y yodo. El área quirúrgica es secada con gasa esteril antes de la realizar la herida. Luego se crea una herida de 8 mm de espesor completo utilizando un punzón de tejido Keyes. Inmediatamente después de realizar la herida, la piel circundante es suavemente estirada para eliminar expansión de la herida. Las heridas son dejadas abiertas durante la duración del experimento. La aplicación del tratamiento es dada de manera tópica durante 5 días comenzando el día de la realización de la herida. Antes del tratamiento, las heridas son limpiadas suavemente con solución salina esteril y esponjas de gasa.

Las heridas son examinadas de manera visual y fotografiadas a una distancia fija el día de la cirugía y a intervalos de dos días de ahí en adelante. El cierre de la herida es determinado mediante medición diaria los días 1-5 y el día 8. Las heridas son medidas de manera horizontal y vertical utilizando un compas de interiores Jameson. Las heridas se consideran curadas si el tejido de granulación ya no es visible y la herida es cubierta por un epitelio continuo.

Se administra una proteína de fusión de albúmina del invento utilizando un rango de dosificación diferente entre 4mg y 500mg por herida por día durante 8 días en vehículo. Los grupos de control de vehículo recibieron 50 mL de solución de vehículo.

Los animales son sacrificados el día 8 con una inyección intraperitoneal de pentobarbital de sodio (300 mg/kg). Las heridas y piel circundante son luego cosechadas para histología e inmunohistoquímica. Los especímenes de tejido son colocados en formalina amortiguada neutral al 10% en casetes de tejido entre esponjas de biopsia para procesamiento adicional.

Tres grupos de diez animales cada uno (5 controles diabéticos y 5 no diabéticos) son evaluados: 1) control de placebo de vehículo, 2) grupo no tratado, y 3) grupo tratado.

El cierre de herida es analizado al medir el área en el eje vertical y horizontal y obtener el área cuadrada total de la herida. Luego la contracción es estimada al establecer las diferencias entre el área inicial de la herida (día 0) y aquella después de tratamiento (día 8). El área de la herida el día 1 es 64 mm2, el tamaño correspondiente de la herida dérmica. Los calculos son hechos utilizando la siguiente fórmula:

a. [área abierta el día 8] - [área abierta el día 1]/[área abierta el día 1]

Los especímenes son fijados en formalina amortiguada al 10% y bloques incrustados de parafina son seccionados perpendiculares a la superficie de la herida (5 mm) y cortados utilizando un microtomo Reichert-Jung. Teñido común de hematoxilina-eosina (H&E) es realizado sobre secciones transversales de heridas divididas. Se utiliza examen histológico de la heridas para evaluar si el proceso de curación y la apariencia morfológica de la piel reparada es alterada con el tratamiento con una proteína de fusión de albúmina del invento. Esta evaluación incluyó verificación de la presencia de acumulación de células, células inflamatorias, capilaridades, fibroblastos, re-epitelialización y madurez epidérmica (Greenhalgh, D. G. *Am. J. Pathol. 136*:1235 (1990)). Un micrometro de lentes calibrados es utilizado por un observador ciego.

Las secciones de tejido también son teñidas inmunohistoquimicamente con un anticuerpo de queratina anti-humano de conejo policlonal utilizando un sistema de detección ABC Elite. Se utiliza piel humana como un control de tejido positivo mientras IgG no inmune se utiliza como un control negativo. El crecimiento de queratinocitos es determinado mediante evaluación de la medida de reepitelialización de la herida utilizando un micrometro de lentes calibrado.

La proliferación de antigenos/ciclina nuclear celular (PCNA) en especímenes de piel es demostrada mediante utilización de anticuerpo anti-PCNA (1:50) con un sistema de detección ABC Elite. Cáncer de colon humano sirvió como un control de tejido positivo y el tejido cerebral humano es utilizado como un control de tejido negativo. Cada especímen incluyó una secció con omisión del anticuerpo primario y sustitución con IgG de ratón no inmune. La clasificación de estas secciones se base en la medida de proliferación a una escala 0-8, el lado inferior de la escala reflejando proliferación leve al lado superior reflejando proliferación intensa.

Los datos experimentales son analizados utilizando una prueba t unpaired. Un valor p de < 0.05 es considerado significativo.

Modelo de Rata Deteriorado por Esteroides

La inhibición de curación de heridas por esteroides ha sido bien documentada en varios sistemas *in vitro* y *in vivo* (Wahl, Glucocorticoides y Curación de Heridas. En: Acción Esteroide Anti-inflamatoria: Aspectos Básicos y Clínicos. 280-302 (1989); Wahlet, *J. Immnunol. 115:* 476-481 (1975); Werb, *J. Exp. Med. 147:* 1684-1694 (1978)). Los glucocorticoides retardan la curación de heridas al inhibir angiogénesis, disminuir la permeabilidad vascular (Ebert, *An. Intern. Med.* 37: 701-705 (1952)), proliferación de fibroblastos, y síntesis de colegeno (Beck, *Factores de Crecimiento.* 5: 295-304 (1991); Haynes, *J. Clin. Invest. 61:* 703-797 (1978)) y producción de una reducción temporal en monocitos circulantes (Haynes, *J. Clin. Invest. 61:* 703:797 (1978); Wahl, Glucocorticoides y curación de heridas", En: Acción Esteroide Antiinflamatoria: Aspectos Básicos y Clinicos, Academic Press, New York, pp. 280-302 (1989)). La administración sistemática de esteroides de curación deteriorada de heridas es una fenómeno bien establecido en ratas (Beck, *Factores de crecimiento.* 5: 295-304(1991); Haynes *J. Clin. Invest. 61:* 703-797 (1978); Wahl, "glucocorticoides y curación de heridas"; En: Acción Esteroide Antiinflamatoria: Aspectos Básicos y Clínicos, Academic Press, New York, pp. 280-302 (1989); Pierce *Proc. Natl. Acad. Sci USA 86:* 2229-2233 (1989)).

Para demostrar que una proteína de fusión de albúmina del invento puede acelerar el proceso de curación, se evaluan los efectos de múltiples aplicaciones tópicas de la proteína de fusión sobre heridas de piel excisional de espesor completo en ratas en las cuales la curación ha sido deteriorada mediante la administración sistémica de metilprednisolona.

Ratas macho adultas jovenes Sprague Dawley que pesan entre 250-300 g (Charles River Laboratories) se utilizan en este ejemplo. Los animales son comprados a ocho semanas de edad y tienen 9 semanas de edad al comienzo del estudio. La respuesta de curación de ratas es deteriorada por la administración sistémica de metilprednisolona (17 mg/kg/rata de manera intramuscular) en el momento de la realización de la herida. Los animales son albergados individualmente y recibieron alimento y agua sin restricción. Todas las manipulaciones son realizadas utilizando técnicas asépticas. Este estudio es realizado según las normas y pautas de Human Genoma Sciences, Inc. Comite Institucional de Cuidado y Uso Animal y las Pautas para el Cuidado y Uso de Animales de Laboratorio.

El protocolo de curación es seguido según aquel descrito anteriormente. El día de la realización de la herida, los animales son anestesiados con una inyección intramuscular de cetamina (50 mg/kg) y xilazina (5 mg/kg). La región dorsal del animal es afeitada y la piel es lavada con 70% etanol y soluciones de yodo. El área quirúrgica es secada con gasa estéril antes de realizar la herida. Una herida de espesor completo de 8 mm es creada utilizando un punzón de tejido Keyes. Las heridas son dejadas abiertas durante la duración del experimento. Las aplicaciones de materiales de prueba son dadas de manera tópica una vez por día durante 7 días consecutivos comenzando el día de la realización de la herida y luego de administración de

metilprednisolona. Antes de tratamiento, las heridas son limpiadas suavemente con solución salina estéril y espongas de gasa.

Las heridas son examinadas de manera visual y fotografiadas a una distancia fija el día de la realización de la herida y al final del tratamiento. El cierre de la herida es determinado mediante medición diaria los días 1-5 y el día 8. Las heridas son medidas de manera horizontal y vertical utilizando un compás de interiores Jameson calibrado. Las heridas son consideradas curadas si el tejido de granulación ya no es visible y la herida es cubierta por epitelio continuo.

La proteína de fusión del invento es administrada utilizando un rango de dosis diferentes, entre 4mg y 500 mg por herida por día durante 8 días en vehículo. Los grupos de control de vehículo recibieron 50 mL de solución de vehículo.

Los animales son sometidos a eutanasia el día 8 con una inyección intraperitoneal de pentobarbital de sodio (300 mg/kg). Las heridas y piel circundante son luego cosechadas para histología. Los especimenes de tejidos son colocados en formalina amortiguada neutral al 10% en casetes de tejido entre esponjas de biopsia para procesamiento adicional.

Tres grupos de 10 animales cada uno (5 con metilprednisolona y 5 sin glucocorticoide) son evaluados: 1) grupo sin tratar 2) control placebo de vehículo 3) grupos tratados.

El cierre de la herida es analizado al medir el área en el eje vertical y horizontal y obtener el área total de la herida. Luego el cierre es estimado al establecer las diferencias entre el área de herida inicial (día 0) y aquella luego del tratamiento (día 8). El área de la herida el día 1 es 64 mm2, el tamaño correspondiente de la herida dérmica. Los calculos son hechos utilizando la siguiente fórmula:g

b. [área abierta el día 8] - [área abierta el día 1]/ [área abierta el día 1]

Los especímenes son fijados en formalina amortiguada al 10% y los bloques incrustados son seccionados perpendiculares a la superficie de herida (5mm) y cortados utilizando un microtomo Olympus. Teñido común de hematoxilina-eosina (H&E) es realizado en secciones transversales de heridas divididas. El examen histológico de las heridas permite evaluar si el proceso de curación y la apariencia morfológica de la piel reparada es mejorada por el tratamiento con una proteína de fusión de albúmina del invento. Se utilizó un microtítulo de lentes calibrado por un observador para determinar la distancia de la abertura de la herida.

Los datos experimentales son analizados utilizando una prueba t unpaired. Un valor p de < 0.05 es considerado significativo.

EJEMPLO 22: Modelo Animal de Linfedema

El propósito de este enfoque experimental es crear un modelo de linfedema apropiado y consistente para probar los efectos terapéuticos de una proteina de fusión de albúmina del invento en linfangiogenesis y re-establecimiento del sistema circulatorio linfático en la extremidad trasera de la rata. La efectividad es medida por el volumen de hinchazón de la extremidad afectada, cuantificación de la cantidad de vasculatura linfática, proteína de plasma sanguineo total, e histopatología. Se observó linfedema agudo durante 7-10 días. Quizas más importante, el progreso crónico del edema es seguido durante hasta 3-4 semanas.

Antes de comenzar la cirugía, se extrae una muestra de sangre para análisis de concentración de proteína. Ratas macho con peso aproximado de 350 g son dosificadas con Pentobarbital. Luego, las piernas derechas son rasuradas desde la rodilla a la cadera. El área rasurada es limpiada con gasa empapada en EtOH al 70%. Se extrae sangre para prueba de proteínas total en suero. Las mediciones volumétricas y de circunferencia son hechas antes de inyectar tinte en patas después de marcar dos niveles de medición (0.5 cm por encima del talón, en el punto medio de la pata dorsal). El dorso intradermico de ambas patas derecha e izquierda es

inyectado con 0.05 ml de azul de Evan al 1%. Luego las mediciones volumétricas y de circunferencia son hechas después de inyección de tinte en las patas.

Utilizando la rodilla como una marca, se realiza una incisión inguinal a mitad de la pierna de manera circunferencial permitiendo la localización de los vasos femorales. Luego los principales vasos linfáticos en esta área son coagulados de manera eléctrica o ligados por sutura.

Utilizando una microscopio, los musculos en la parte posterior de la pierna (cerca de semitendinosis y aductores) son divididos contundentemente. Luego se localiza el nódulo linfático popliteal. Los 2 vasos linfáticos proximales y 2 distales y el suministro de sangre del nódulo popliteal son luego ligados por sutura. El nódulo linfático popliteal, y cualquier tejido adiposo acompañante, es luego retirado al cortar tejidos conectivos.

Se debe tener cuidado para controlar cualquier sangrado leve que resulta de este procedimiento. Después de ocluir los linfáticos, los pliegues de piel son sellados utilizando piel líquida (Vetbond) (AJ Buck). Los bordes de piel separados son sellados al tejido muscular subyacente mientras se deja una abertura de 0.5 cm aproximadamente alrededor de la pierna. La piel también puede ser anclada al suturar a musculo subyacente cuando es necesario.

Para evitar infección, los animales son albergados individualmente con malla (sin paja). Los animales en recuperación son revisados diariamente a través del pico edematoso óptimo, el cual ocurrió típicamente el día 5-7. Para evaluar la intensidad del linfedema, se midió la circunferencia y volumenes de 2 sitios designados sobre cada pata antes de la operación y diariamente durante 7 días. El efecto de proteínas de plasma sobre linfedema es determinado y si el análisis de proteínas es un parámetro de prueba útil también es investigado. Los pesos de ambas extremidades de control y edematosas son evaluados en dos sitios. El análisis es realizado en una forma a ciegas.

Mediciones de circunferencia: bajo anestésico de gas breve para prevenir movimiento de extremidades, se utiliza una cinta de tela para medir la circunferencia de extremidades. Las mediciones son hechas en el tobillo y pata dorsal por dos personas diferentes y las dos lecturas son promediadas. Las lecturas son tomadas de ambas extremidades de control y edematosa.

Mediciones volumétricas: El día de la cirugía, los animales son anestesiados con Pentobarbital y son probados antes de la cirugía. Para mediciones volumétricas diarias los animales están bajo anestésico de halotano breve (rápida inmovilización y rápida recuperación), y ambas piernas son rasuradas y marcadas de manera igual utilizando marcador a prueba de agua. Primero las piernas son sumergidas en agua, luego sumergidas en el instrumento hasta cada nivel marcado, luego se hace la medición con un software de edema Buxco (Chen/Victor). Los datos son recuperados por una persona, mientras el otro está sumergiendo la extremidad hasta el área marcada.

Mediciones de proteínas de plasma sanguineo: La sangre es extraida, centrifugada, y el suero es separado antes de cirugía y luego al terminar para comparación total de proteínas y Ca2+.

Comparación de Peso de Extremidades: Después de extraer sangre, el animal es preparado para recolección de tejidos. Las extremidades son amputadas utilizando guillotina, luego ambas piernas experimental y de control son cortadas en la ligadura y pesadas. Un segundo pesaje es hecho al desarticular la articulación tibio-cacaneal y el pie es pesado.

Preparaciones Histológicas: El musculo transverso localizado detrás del área de la rodilla (popliteal) es disecado y dispuesto en un molde metálico, llenado con freezeGel, sumergido en metilbutano frio, colocado en bolsas de muestra rotuladas a -80EC hasta seccionar. Al seccionar, el músculo es observado bajo microscopia fluorescente para linfaticos.

EJEMPLO 23: Eliminación de Expresión de Moléculas de Adhesión Inducida por TNF alfa Mediante una Proteína de Fusión de Albúmina del Invento.

El reclutamiento de linfocitos a áreas de inflamación y angiogénesis involucra interacciones específicas de receptores-ligandos entre moléculas de adhesión de superficie celular (CAMs) sobre linfocitos y el endotelio vascular. El proceso de adhesión , en ambas configuraciones normal y patológica, sigue una cascada de múltiples etapas que involucra expresión de moleculas-1 de adhesión intercelular (CAMP-1), moléculas-1 de adhesión de células vasculares (VCAM-1), moleculas-1 de adhesión de leucocitos endoteliales (E-selectina) sobre células endoteliales (EC). La expresión de estas moléculas y otras sobre el endotelio vascular determina la eficiencia con la cual los leucocitos pueden adherirse a la vasculatura local y extravasarse hacia el tejido local durante el desarrollo de una respuesta inflamatoria. La concentración local de citoquinas y factor de crecimiento participan en la modulación de la expresión de estas CAMs.

El factor de necrosis tumoral alfa (TNF-a), una citoquina proinflamatoria potente, es un estimulador de todas las tres CAMs sobre células endoteliales y puede estar involucrado en una amplia variedad de respuestas inflamatorias, a menudo resultando en un resultado patológico.

El potencial de una proteína de fusión de albúmina para mediar una eliminación de expresión CAM inducida por TNF-a se puede examinar. Un ensayo ELISA modificado el cual utiliza ECs como un absorbente de fase sólida es empleado para medir la cantidad de expresión CAM sobre ECs tratados con TNF-a cuando se co-estimulan con un miembro de la familia FGF de proteínas.

Para realizar el experimento, se obtienen cultivos de células endoteliales de vena umbilical humana (HUVEC) a partir de productos de cordón agrupado y se mantienen en medio de crecimiento (EGM-2; Clonetics. San Diego, CA) suplementados con FCS al 10% y 1% penecilina/estreptomicina en un incubador humidificado a 37 grados C conteniendo CO2 al 5%. Las HUVECs son sembradas en placas de 96 pozos a concentraciones de 1 x 104 células por pozo en medio EGM a 37 grados C durante 18-24 horas o hasta clonfluencia. Las monocapas son subsecuentemente lavadas 3 veces con una solución libre de suero de RPMI-1640 suplementada con 100 U/ml penicilina y 100 mg/ml estreptomicina, y tratadas con una citoquina dada y/o factor de crecimiento durante 24 h a 37 grados C. Después de incubación, las células son evaluadas para expresión CAM.

Las células endoteliales de vena umbilical humana (HUVECs) son cultivadas en una placa de 96 pozos estándar hasta confluencia. El medio de crecimiento de retirado de las células y reemplazado con 90 ul de Medio 199 (FBS al 10%). Muestras para prueba y controles positivos y negativos son agregados a la placa en triplicado (en volumenes de 10 ul). Las placas son incubadas a 37 grados C durante ya sea 5 h (expresión de selectina e integrina) o 24 h (expresión de integrina solamente). Las placas son aspiradas para remover medio y 100 ul de 0.1% paraformaldehido-PBS (con Ca++ y Mg++) se agrega a cada pozo. Las placas son mantenidas a 4°C durante 30 min.

Luego el fijador es retirado de los pozos y los pozos son lavados IX con PBS(+Ca,Mg)+0.5% BSA y drenados. No permitir a los pozos secarse. Agregar 10 ul de anticuerpo primario diluido a los pozos de prueba y control. Anti-IVAM-1-Biotina, Anti-VCAM-1-Biotina y Anti-E-selectina-Biotina se utilizan a una concentración de 10ug/ml (dilución 1:10 de 0.1 mg/ml anticuerpo concentrado). Las células son incubadas a 37°C durante 30 minutos en un ambiente humedecido. Los pozos son lavados X3 con PBS(+Ca, Mg)+0.5%BSA.

Luego agregar 20 ul de ExtrAvidin-Fosfato Alcalino diluido (dilución 1:5.000) a cada pozo e incubar a 37°C durante 30 min. Los pozos son lavados X3 con PBS(+Ca, Mg)+0.5% BSA.

Una tableta de Fosfato p-Nitrofenol pNPP es disuelta en 5 ml de amortiguador de glicina (pH 10.4). 100 ul de sustrato de pNPP en amortiguador de glicina es agregado a cada pozo de prueba. Pozos estándares en triplicado son preparados a partir de la dilución de trabajo de ExtrAvidin-Fosfatosa Alcalina en amortiguador de glicina: 1:5000 (100) > 10 -0.5 > 10-t > 10-1.5. 5 ul de cada dilución son agregados a pozos triplicados y el contenido AP resultante en cada pozo es 5.50 ng, 1.74 ng,g 0.55 ng, 0.18 ng. 100 ul de reactivo pNNP debe ser agregado a cada uno de los pozos estándares. La placa debe ser incubada a 37°c durante 4h. Un volumen de 50 ul de 3M NaOH es agregado a todos los pozos. Los resultados son cuantificados sobre un lector de placa a 405 nm. La opción de resta de entorno se utiliza sobre pozos vacíos llenados con amortiguador de glicina solamente. El modelo es configurado par indicar la concentración de AP-conjugado en cada pozo estándar [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Los resultados son indicados como cantidad de conjugado AP unido en cada muestra.

EJEMPLO 24: Construcción de Constructo Reportero GAS

Una ruta de transducción de señales involucrada en la diferenciación y proliferación de células es llamada la ruta Jaks-STATs. Las proteínas activadas en la ruta Jacks-STATs se unen a elementos "GAS" de sitios de activación gama o elemento responsivo sensitivo a interferón ("ISRE"), localizados en el promotor de muchos genes. La unión de una proteína a estos elementos altera la expresión del gen asociado.

Los elementos GAS e ISRE son reconocidos por una clase de factores de transcripción llamados Transductores de Señal y Activadores de Transcripción, o "STATs". Existen seis miembros de la familia STATs. Stat1 y Stat3 están presentes en muchos tipos celulares, como es Stat2 (cuando la respuesta a IFN-alfa es diseminada). Stat4 es más restringido y no está en muchos tipos celulares aunque se ha encontrado en células auxiliares T Clase I después de tratamiento con IL-12. Stat5 originalmente fue llamado factor de crecimiento mamario, pero se ha encontrado en concentraciones mayor en otras células incluyendo células mieloides. Este se puede activar en células de cultivo de tejidos por muchas citoquinas.

Los STATs son activados para translocar desde el citoplasma hasta el nucleo ante fosforilación de tirosinas mediante un grupo de quinasas conocidas como la familia de Quinasas Janus ("Jacks"). Las jacks representan una familia distinta de tirosina-quinasas solubles e incluyen Tyk2, Jak1, y Jak3. Estas quinasas presentan similitud de secuencia significativa y generalmente son catalíticamente inactivas en células en reposo.

Las Jacks son activadas por un rango amplia de receptores resuminados en la Tabla a continuación. (Adaptado de la reseña de Schidler y Darnell, Ann. Rev. Biochem. 64:621-51 (1995)). Una familia de receptores de citoquinas, capaz de activar Jacks, es dividida en dos grupos: (a) Clase 1 incluye receptores para IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, y trombopoyetina; y (b) la Clase 2 incluye IFN-a, IFN-g, y IL-10. Los receptores de clase 1 comparten un motivo de cisteina conservado (un grupo de cuatro cisteinas conservadas y un triptofan) y un motivo WSXW (una región proximal de membrana que codifica Trp-Ser-Xaa-Trp-Ser (SEQ ID NO:53)).

Por lo tanto, al unir un ligando a un receptor, las Jacks son activadas, lo cual a su vez activan STATs, que luego translocan y unen a elementos GAS. Este proceso completo es contemplado en la ruta de transducción de señales Jaks-STATs. Por lo tanto, la activación de la ruta Jacks-STATs, reflejada por la unión del elemento GAS o ISRE, se puede utilizar para indicar proteínas involucradas en la proliferación y diferenciación de células. Por ejemplo, los factores de crecimiento y citoquinas son conocidos por activar la ruta Jacks-STATs (ver Tabla 5, a continuación). Por lo tanto, al utilizar elementos GAS ligados a moléculas reporteras, los activadores de la ruta Jacks-STATs se pueden identificar.

TABLA 5

JAKs		ESTADISTICA			GAS(elementos) o ISRE	
LiGANDO		tyk2	Jak1	Jak2	Jak3	
Famiilia IFN						
IFN-a/B	+	+	-	-	1,2,3	ISRE
IFN-g		+	+	-	GAS(IRF1>Lys6>IFP)	
11-10	+	?	?	-	1,3	
Familia gp 130						
IL-6(pleiotropica)	+	+	+	?	1.3	GAS(IRFI>Lys6>IFP)
11-11(pleiotropica)	¿	+	¿	¿	1,3	
OnM((pleiotropica)	¿	+	+	¿	1,3	
LIF(pleiotropica)	¿	+	+	¿	1.3	
CNTF(pleiotropica)	-/+	+	+	¿	1,3	
G-CSF(pleiotropica)	¿	+	¿	¿	1,3	
IL-12(pleiotropica)	+	-	+	+	1,3	
Familia g-C						
IL-2 (Linfocitos)	-	+	-	+	1,3,5	GAS
IL-4 (lifocítica/mieloide)-GAS(IRF1=IFP>Ly6)		+	-	+	6	(lgH)
IL-7 (linfocitos)	-	+	-	+	5	GAS
IL-9 (limfocitos)	-	+	-	+	5	GAS
Il-13 (limfocitos)	-	+	¿	¿	6	GAS
IL-15	¿	+	¿	+	5	GAS
Familia gp 140						
IL-3 (mieloide)	-	-	+	-	5	GAS(IFF1>IFP>>Ly6)
IL-5(mieloide)	-	-	+	-	5	GAS
GM-CSF (miloide)	-	-	+	-	5	GAS
Familia de la hormona de crecimiento						
GH	?	-	+	-	5	
PRL	?	+/-	+	-	1,3,5	
EPO	?	-	+	-	5	GAS(B-CAS>IRF1= Ly6)
Tirosinaquinasas Receptoras						
EGF	?	+	+	-	1,3	GAS(IRF1)
PDGF	?	+	+	-	1,3	
CSF-1	?	+	+	-	1,3	GAS(not IRF1)

Para construir un GAS sintético que contenga el elemento promotor, el cual se utiliza en los Ensayos Biológicos descritos en los Ejemplos 27-29, se emplea una estrategia basada en PCR para generar una secuencia promotora GAS-SV40. El cebador 5' contiene cuatro objetos tandem del sitio de unión GAS encontrados en el promotor IRF1 y previamente demostrados por unir

STATs ante inducción con un rango de citoquinas (Rothman, Immunity 1:457-468 (1994).), aunque otros elementos GAS o ISRE se pueden utilizar. El cebador 5' también contiene 18bp de complementariedad de secuencia a la secuencia promotora temprana SV40 y es flanqueado con un sitio XhoI. La secuencia del cebador 5' es: 5´-

GCGCCTCGAGATTTCCCCGAAATCTAGATTTCCCCCGAAATCTAGATTTCCCCGAAAT GATTTCCCCGAAATGATTTCCCCGAAATATCTGCCATCTCAATTAG: 3´-(SEQ ID NO:54)

El cebador corriente abajo es complementario con el promotor SV40 y es flanqueado con un sitio Hind III:

5':GCGGCAAGCTTTTTGCAAAGCCTAGGC: 3' (SEQ ID NO:55)

La amplificación PCr es realizada utilizando el modelo promotor SV40 presente en el plásmido B-gal:promotor obtenido en Clontech. El fragmento PCR resultante es digirido con XhoI/hind III y subclonado a BLSK2. (Stratagene). El secuenciamiento con cebadores delanteros y inversos confirma que el inserto contiene la siguiente secuencia:

5´-
CTCGAGATTTCCCCGAAATCTAGATTTCCCCGAAATGATTTCCCCGAAATGATTTCCC CGAAATATCTTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCC ATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCTGGCTGACTAATTTTT TTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAG GAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTT: 3`(SEQ ID NO:56)

Con este elemento promotor GAS ligado al promotor SV40, luego se diseña un constructo reportero GAS:SEAP2. Aquí, la molécula reportera es una fosfatasa alcalina secretada, o "SEAP." en este o en cualquiera de los otros ejemplos. Moléculas reporteras bien conocidas que se pueden utilizar en cambio de SEAP incluyen acetiltransferasa de cloranfenicol (CAT), luciferasa, fosfatasa alcalina, B-galactosidasa, proteína fluorescente verde (GFP), o cualquier proteína detectable por un anticuerpo.

La anterior secuencia confirmó que el elemento promotor GAS-SV40 sintético es subclonado al vector pSEAP-Promotor obtenido a partir de Clontech utilizando HindIII y XhoI, al reemplazar efectivamente el promotor SV40 con el elemento promotor GAS-SV40 amplificado, para crear el vector GAS-SEAP. Sin embargo, este vector no contiene un gen de resistencia de neomicina, y por lo tanto, no es preferido para sistemas de expresión mamífera.

Por lo tanto, con el objeto de generar líneas celulares estables mamíferas que expresen el reportero GAS-SEAP, el casette GAS-SEAP es retirado del vector GAS-SEAP utilizando SalI y NotI en un vector esqueleto que contiene el gen de resistencia de neomicina, tal como pGFP-1 (Clontech), utilizando estos sitios de restricción en el sitio múltiple de clonación, para crear el vector GAS-SEAP/Neo. Una vez transfectado a células mamíferas, este vector se puede utilizar como una célula reportera para unión GAS como se describe en los Ejemplos 27-29.

Otros constructos se pueden realizar utilizando la anterior descripción y reemplazando GAS con una secuencia promotora diferente. Por ejemplo, la construcción de moleculas reporteras que contienen las secuencias promotoras EGR y NF-KB se describen en los Ejemplos 27-31. Sin embargo, muchos otros promotores se pueden sustituir utilizando los protocolos descritos en estos Ejemplos. Por ejemplo, SRE, IL-2, NFAT, o promotores de Osteocalcina se pueden sustituir solos o en combinación (ej., GAS/NF-KB/EGR, GAS/NF-KB, IL-2/NFAT, o NF-KB/GAS). De manera similar, se pueden utilizar otras líneas celulares para probar actividad de constructo reportero, tal como HELA (epitelial), HUVEC (endotelial), Reh (células B), Saos-2 (osteoblastos), HUVAC (artica), o cardiomiocitos.

EJEMPLO 25: Ensayo para Actividad SEAP.

Como una molécula reportera para los ensayos descritos en la presente, la actividad SEAP es probada utilizando el kit Tropix Phospho-light (Cat. BP-400) según el procedimiento general. El kit Tropix Phospho-light suministra la Dilución, Ensayo, y Amortiguadores de Reacción utilizados a continuación.

Cebar un dispensador un el Amortiguador de Dilución 2.5x y dispensar 15 ul de amortiguador de dilución 2.5x en Optiplacas conteniendo 35 ul de una solución que contiene una proteína de fusión de albúmina del invento. Sellar las placas con un sellador plástico e incubar a 65 grados C durante 30 min. Separar las optiplacas para evitar calentamiento desigual.

Enfriar las muestras a temperatura ambiente durante 15 minutos. Vaciar el dispensador y cebar con el Amortiguador de Ensayo. Agregar 50 ml de Amortiguador de Ensayo e incubar a temperatura ambiente durante 5 minutos. Vaciar el dispensador y cebar con Amortiguador de Reacción (ver Tabla a continuación). Agregar 50 ul de Amortiguador de Reacción e incubar a temperatura ambiente durante 20 minutos. Puesto que la intensidad de la señal quimioluminiscente es dependiente de tiempo, y toma aproximadamente 10 minutos para leer 5 placas sobre un luminometro, por lo tanto se deben tratar 5 placas cada vez y comenzar con el segundo grupo 10 minutos después.

Leer la unidad luminica relativa en el luminometro. Colocar H12 como vacío, e imprimir los resultados. Un aumento en quimioluminiscencia indica actividad reportera.

Tabla 6

# de placas	diluyente amortiguador Rxn (ml)	CSPD (ml)	# de placas	diluyente amortiguador Rxn (ml)	CSPD (ml)
10	60	3	31	165	8.25
11	65	3.25	32	170	8.5
12	70	3.5	33	175	8.75
13	75	3.75	34	180	9
14	85	4	36	185	9.25
15	85	4.25	36	190	9.5
16	90	4.5	37	195	9.75
17	95	4.75	38	200	10
18	100	5	39	205	10.25
19	105	5.25	40	210	10.5
20	110	5.5	41	215	10.75
21	115	5.75	42	220	11
22	120	6	43	225	11.25
23	125	6.25	44	230	11.5
24	130	6.5	45	235	11.75
25	135	6.75	46	240	12
26	140	7	47	245	12.25
27	145	7.25	48	250	12.5
28	150	7.5	49	255	12.75
29	155	7.75	50	260	13
30	160	8			

EJEMPLO 26: Ensayo que Identifica Actividad Neuronal

Cuando las células experimentan diferenciación y proliferación, un grupo de genes son activados a través de muchas rutas diferentes de transducción de señales. Uno de estos genes, EGR1 (gen 1 de respuesta de crecimiento temprano), es inducido en varios tejidos y tipos de células ante activación. El promotor de EGR1 es responsable de tal inducción. Utilizando el promotor EGR1 ligado a las moléculas reporteras, se puede evaluar la capacidad de las proteínas de fusión del invento para activar las células.

Particularmente, el siguiente protocolo se utiliza para evaluar actividad neuronal en líneas celulares PC12. Las células PC12 (células de fenocromocitoma de rata) son conocidas por proliferar y/o diferenciar mediante activación con un número de mitogenos, tales como TPA (acetato de forbol de tetradecanoilo), NGF (factor de crecimiento nervioso), y EGF (factor de crecimiento epidermal). La expresión del gen EGR1 es activada durante este tratamiento. Por lo tanto, al transfectar establemente células PC12 con un constructo que contiene un promotor EGR ligado al reportero SEAP, se puede evaluar la activación de células PC12 mediante una proteína de fusión de albúmina del invento.

El constructo reportero EGR/SEAP se puede ensamblar mediante el siguiente protocolo. La secuencia de promotor EGR-1 (-633 a +1)(Sakamoto, Oncogeno 6:867-871) (1991)) se puede amplificar por PCR a partir de ADN genómico humano utilizando los siguientes cebadores:

Primer cebador: 5`GCGCTCGAGGGATGACAGCGATAGAACCCCGG-3` (SEQ ID NO:57)

Segundo cebador: 5`GCGAAGCTTCGCGACTCCCCGGATCCGCCTC-3`(SEQ ID NO:58)

utilizando el vector GAS:SEAP/Neo producido en el Ejemplo 24, el producto EGR1 amplificado luego se puede insertar en este vector. Linearizar el vector GAS:SEAP/Neo utilizando enzimas de restricción Xho/HindIII, retirando el stuffer GAS/SV40. Restringir el producto EGR1 amplificado con estas mismas enzimas. Ligar el vector y el promotor RGR1.

Para preparar placas de 96 pozos para cultivo celular, se agregan dos mls de una solución de revestimiento (dilución 1:30 de colágeno tipo I (Upstate Biotech Inc. Cat#08-115) en 30% etanol (filtro esterilizado)) por una placa de 10 cm o 50 ml por pozo de la placa de 96 pozos, y se deja secar al aire durante 2 horas.

Las células PC12 son desarrolladas de manera comun en medio RPMI-1640 (Bio Whittaker) conteniendo 10% suero de caballo (JRH BIOSCIENCES, Cat. #12449-78P), 5% de suero bovino fetal inactivado por calor (FBS) suplementado con 100 unidades/ml de penicilina y 100 ug/ml de estreptomicina sobre un disco de cultivo de tejido de 10 cm pre-recubierto. Una a cuatro divisiones es hecha cada tres a cuatro días. Las células son retiradas de las placas mediante raspado y resuspendidas con pipeteo arriba y abajo por más de 15 veces.

Transfectar el constructo EGR/SEAP/Neo a PC12 utilizando técnicas conocidas en el estado de la técnica. Las células estables EGR-SEAP/PC12 son obtenidas al cultivar las células en 300 ug/ml G418. Se utiliza medio libre de G418 para crecimiento de rutina pero cada uno a dos meses, las células deben ser re-cultivadas en 300 ug/ml de G418 para pareja de pasajes.

Para probar la actividad neuronal, una placa de 10 cm con células alrededor de 70 a 80% confluentes es clasificada al retirar el medio viejo. Lavar las células una vez con PBS (solución salina amortiguada por fosfato). Luego despojar las células en medio de suero viejo (RPMI-1640 conteniendo 1%suero de caballo y 0.5% FBS con antibióticos) duante la noche.

A la manaña siguiente, retirar el medio y lavar las células con PBS. Raspar las células de la placa, suspender las células en 2 ml de medio de suero bajo. Contar el numero de células y agregar más medio de suero bajo para alcanzar la densidad celular final como 5x105 células/ml.

Agregar 200 ul de la suspensión de células a cada pozo de placa de 96 pozos (equivalente a 1x105 células/pozo). Agregar una serie de diferentes concentraciones de una proteina de fusión de albúmina del invento, 37 grados C durante 48 a 72 horas. Como un control positivo, se puede utilizar un factor de crecimiento conocido por activar células PC12 a través de EGR, tal como 50 ng/ul de Factor de Crecimiento Neuronal (NGF). Inducción de más de 15 veces de SEAP es vista tipicamente en los pozos de control positivo. El ensayo SEAP puede ser realizado de manera común utilizando técnicas conocidas en el estado de la técnica y/o como se describió en el Ejemplo 25.

EJEMPLO 27: Ensayo para Actividad de Células T.

El siguiente protocolo es utilizado para evaluar la actividad de células T al identificar factores, y determinar si una proteína de fusión de albúmina del invento prolifera y/o diferencia células T. La actividad de células T es evaluada utilizando el constructo GAS/SEAP/Neo producido en el Ejemplo 24. Por lo tanto, los factores que aumentan la actividad SEAP indican la capacidad para activar la ruta de transducción de señales Jaks-STATS. La célula T utilizada en este ensayo es célula T Jurkat (Numero de Acceso ATCC TIB-152), aunque las células Molt-3 (No. de Acceso CRL-1552) y células Molt-4 (Número de Acceso ATCC CRL-1582) también se pueden utilizar.

Las células T Jurkat son células auxiliares Th1 CD4+ linfoblásticas. Con el objeto de generar líneas celulares estables, aproximadamente 2 millones de células Jurkat son transfectadas con el vector GAS-SEAP/neo utilizando DMRIE-C (Life Technologies)(procedimiento de transfección descrito a continuación). Las células transfectadas son sembradas a una densidad de aproximadamente 20.000 células por pozo y transfectantes resistentes a 1 mg/ml genticina seleccionada. Las colonias resistentes son expandidas y luego probadas para su respuesta a concentraciones crecientes de interferón gama. La respuesta de dosis de un clon seleccionado es demostrada.

Especificamente, el siguiente protocolo producirá sufucientes células para 75 pozos conteniendo 200 ul de células. Este es realizado ya sea de manera progresiva o en series múltiples para generar suficientes células para multiples placas de 96 pozos. Las células Jurkat son mantenidas en RPMI + suero al 10% con 1% Pen-Strep. Combinar 2.5 mls de OPTI-MEM (Life Tecnologies) con 10 ug de ADN plásmido en un frasco T25. Agregar 2.5 ml de OPTI-MEM conteniendo 50 ul de DMRIE-C e incubar a temperatura ambiente duante 15-45 min.

Durante el periodo de incubación, contar la concentración de células, centrifugar el número requerido de células (10 7 por transfección), y resuspender en OPTI-MEM hasta una concentración final de 10 7 células/ml. Luego agregar 1 ml de 1 x 10 7 células en OPTI-MEM a un frasco T25 e incubar a 37 grados C durante 6 horas. Después de incubación, agregar 10 ml de RPMI + suero al 15%.

Las líneas reporteras estables Jurkat:GAS-SEAP son mantenidas en RPMI + 10% suero, 1 mg/ml Genticina, y 1% Pen-Strep. Estas células son tratadas con concentraciones variables de una o más proteínas de fusión del presente invento.

El día del tratamiento con la proteína de fusión, las células se deben lavar y suspender en RPMI fresco + 10% suero a una densidad de 500.000 células por ml. El número exacto de células requeridas dependerá del número de proteínas de fusión en el número de concentraciones diferentes de proteínas de fusión seleccionadas. Para una placa de 96 pozos, aproximadamente 10 millones de células (para 10 placas, 100 millones de células) son requeridas.

Los platos de pozos que contienen células Jurkat tratadas con la proteína de fusión son colocados en un incubador durante 48 horas (nota: este tiempo es variable entre 48-72 horas). Muestras de 35 ul de cada pozo son luego transferidas a una placa de 96 pozos opaca utilizando una pipeta de 12 canales. Las placas opacas se deben cubrir (utilizando cubiertas de celofan) y almacenar a -20 grados C hasta que los ensayos SEAP sean realizados según el Ejemplo 25. Las placas que contienen las células tratadas restantes son colocadas a 4 °C y sirven como una fuente de material para repetir el ensayo sobre un pozo especifico si se desea.

Como un control positivo, se puede utilizar 100 Unidades/ml de interferón gama la cual es conocida por activar células Jurkat T. Tipicamente se observa una inducción de 30 veces en los pozos de control positivo.

El anterior protocolo se puede utilizar en la generación de células transfectadas temporales así como estables, las cuales serían manifiestas a aquellos con experiencia en el estado de la técnica.

EJEMPLO 28: Ensayo para Actividad de Células T.

NF-KB(Factor Nuclear KB) es un factor de transcripción activado mediante una amplia variedad de agentes incluyendo las citoquinas inflamatorias IL-1 y TNF, CD30 y CD40, linfotoxina-alfa y linfotoxina-beta, mediante exposición a LPS o trombina, y mediante expresión de cuertos productos genéticos virales. Como un factor de transcripción, NF-KB regula la expresión de genes involucrada en activación de células inmunes, control de apoptosis (NF-KB parece proteger las células de apoptosis), desarrollo de células B y T, respuestas anti-virales y antimicrobianas, y múltiples respuestas de estrés.

En condiciones no-estimuladas, NF-KB es retenido en el citoplasma con I-KB (Inhibidor KB). Sin embargo, ante estimulación, I-KB es fosforilado y degradado, haciendo que NF-KB se mueva hacia el núcleo, activando así la transcripción de genes objetivo. Los genes objetivo activados por NF-KB incluyen IL-2, IL-6, GM-CSF, ICAM-1 y MHC clase 1.

Debido a su rol central y capacidad para responder a un rango de estimulos, los constructos reporteros que utilizan el elemento promotor NF-KB se utilizan para seleccionar la proteína de fusión. Los activadores o inhibidores de NF-KB serían útiles para tratar, prevenir, y/o diagnosticar enfermedades. Por ejemplo, se podrían utilizar inhibidores NF-KB para tratar aquellas enfermedades relacionadas con la activación aguda o crónica de NF-KB, tal como artritis reumatoide.

Para construir un vector que contiene el elemento promotor NF-KB, se emplea una estrategia basada en PCR. El cebador corriente arriba contiene cuatro copias en tándem del sitio de unión NF-KB (GGGGACTTTCCC) (SEQ ID NO:59), 18 bp de la secuencia complementaria al extremo 5' de la secuencia promotora temprana SV40, y es flanqueado con un sitio XhoI:

5`:
GCGGCCTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGACTTTCCATC
CTGCCATCTCAATTAG: (SEQ ID NO:60)

5`-GCGGCAAGCTTTTTGCAAAGCCTAGGC:3′ (SEQ ID NO:55)

5′:CTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGACTTTCCATCTGCC
ATCTCAATTAGTCAGCAACCATAGTCCGCCCCTAACTCCGCCCATCCCGCCCCCTAAC
TCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAG
AGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGG
AGGCCTAGGCTTTTGCAAAAAGCTT:3′ (SEQ ID NO: 61)

Luego, reemplazar el elemento promotor mínimo SV40 presente en el plásmido promotor pSEAP2 (Clontech) con este fragmento NF-KB/SV40 utilizando XhoI y HindIII. Sin embargo,

este vector no contiene un gen de resistencia a neomicina, y por lo tanto, no es preferido para sistemas de expresión mamífera.

Con el objeto de generar líneas de células mamíferas estables, el casete NF-KB/SV40/SEAP es retirado del anterior vector NF-KB/SEAP utilizando enzimas de restricción SalI y NotI, e insertado en un vector que contiene resistencia de neomicina. Particularmente, el casete NF-KB/SV40/SEAP fue insertado en pGFP-1 (Clontech), reemplazando el gen GFP, después de restringir pGFP-1 con SalI y NotI.

Una vez creado el vector NF-KB/SV40/SEAP/Neo, las células Jurkat estables son creadas y mantenidas según el protocolo descrito en el Ejemplo 25. De manera similar, el método para probar las proteínas de fusión con estas células T Jurkat también es descrito en el Ejemplo 25. Como un control positivo, TNF alfa exógeno (0.1, 1, 10 ng) es agregado a los pozos H9, H10, y H11, con una activación de 5-10 veces típicamente observada.

EJEMPLO 29: Ensayo que Identifica Actividad Mieloide.

El siguiente protocolo se utiliza para evaluar la actividad mieloide de una proteína de fusión de albúmina del presente invento al determinar si la proteína de fusión prolifera y/o diferencia células mieloides. La actividad celular mieloide es evaluada utilizando el constructo GAS/SEAP/Neo producido en el Ejemplo 24. Por lo tanto, los factores que aumentan la actividad SEAP indican la capacidad para activar la ruta de transducción de señales Jaks-STATS. La célula mieloide utilizada en este ensayo es U937, una línea de células de pre-monocitos, aunque TF-1, HL60, o KG1 se pueden utilizar.

Para transfectar temporalmente U937 con el constructo GAS/SEAP/Neo producido en el Ejemplo 24, se utiliza un método DEAE-Dextran (Kharbanda, 1994, Crecimiento Celular & Diferenciación, 5:259-265). Primero, cosechar 2x10 7 células U937 y lavar con PBS. Las células U937 usualmente son cosechadas en medio RPMI 1640 conteniendo 10% suero bovino fetal inactivado por calor (FBS) suplementado con 100 unidades/ml penicilina y 100 mg/ml de estreptomicina.

Luego, suspender las células en 1 ml de amortiguador 20 mM Tris-HCl (pH 7.4) conteniendo 0.5 mg/ml DEAE-Dextran, 8 ug de ADN plásmido GAS-SEAP2, 140 mM NaCl, 5 mM KCl, 375 uM Na2HPO4./H2O, 1 mM MgCl2, y 675 uM CaCl2. Incubar a 37 grados C durante 45 min.

Lavar las células con medio RPMI 1640 conteniendo FBS al 10% y luego resuspender en 10 ml de medio completo e incubar a 37 grados C durante 36 horas.

Las células estables GAS-SEAP/U937 son obtenidas al cultivar lás células en 400 ug/ml G418. Se utiliza medio libre de G418 para crecimiento de rutina pero cada uno a dos meses, las células se deben re-cultivar en 400 ug/ml de G418 para pareja de pasajes.

Estas células son probadas al cosechar 1x10 8 células (esto es suficiente para ensayo de 10 placas de 96 pozos) y lavar con PBS. Suspender las células en 200 ml del medio de crecimiento antes descrito, con una densidad final de 5x10 5 células/ml. Sembrar 200 il de células por pozo en la placa de 96 pozos (o 1x10 5 células/pozo).

Agregar diferentes concentraciones de la proteína de fusión. Incubar a 37 grados C durante 48 a 72 h. Como un control positivo, se puede utilizar 100 unidades/ml de interferón alfa la cual es conocida por activar U937 células. Tipicamente se observa una inducción mayor de 30 veces en los pozos de control positivo. Probar mediante ensayo SEAP el sobrenadante según los métodos conocidos en el estado de la técnica y/o el protocolo descrito en el Ejemplo 25.

EJEPLO 30: Ensayo para Identificar Cambios en Concentración de Moléculas Pequeñas y Permeabilidad de Membrana.

Se sabe que la unión de un ligando a un receptor altera los niveles intracelulares de moléculas pequeñas, tales como calcio, potasio, sodio, y pH, así como también el potencial de membrana. Estas alteraciones se pueden medir en un ensayo para identificar proteínas de fusión las cuales se unen a receptores de una célula particular. Aunque el siguiente protocolo describe un ensayo para calcio, este protocolo se puede modificar facilmente para detectar cambios en potasio, sodio, pH, potencial de membrana o cualquier otra molécula pequeña que es detectable mediante una sonda fluorescente.

El siguiente ensayo utiliza Lector de Placas de Imágenes Fluorométricas ("FLIPR") para medir cambios en moléculas fluorescentes (Sondas Moleculares) que fijan moleculas pequeñas. Claramente, cualquier molécula fluorescente que detecta una molecula pequeña se puede utilizar en cambio de la molecula fluorescente de calcio, aquí se utilizó fluo-4 (Sondas Moleculares, Inc.; catalogo No. F-14202).

Para células adherentes, sembrar las células a 10.000 - 20.000 células/pozo en una placa de 96 pozos Co-star Black con fondo claro. La placa es incubada en un incubador de CO2 durante 20 horas. Las células adherentes son lavadas dos veces en un lavador Biotek con 200 ul de HBSS (Solución de Sal Balanceada de Hank) dejando 100 ul de amortiguador después de la lavada final.

Una solución concentrada de 1 mg/ml de fluo-4 es hecha en 10% ácido plurónico DMSO. Para cargar las células con fluo-4, se agregan 50 ul de 12 ug/ml de fluo-4 a cada pozo. La placa es incubada a 37 grados C en un incubador CO2 durante 60 minutos. La placa es lavada cuatro veces en la lavadora Biotek con HBSS dejando 100 ul de amortiguador.

Para células no adherentes, las células son centrifugadas a partir de medio de cultivo. Las células son re-suspendidas a 2-5x10 6 células/ml con HBSS en un tubo conico de 50 ml. Se agregó 4 ul de 1 mg/ml solución fluo-4 en 10% ácido plurónico DMSO a cada ml de suspensión celular. Luego el tubo es colocado en un baño de agua a 37 grados C durante 30-60 min. Las células son lavadas dos veces con HBSS, resuspendidas a 1 x10 6 células/ml, y dispensadas en una microplaca, 100 ul/pozo. La placa es centrifugada a 1000 rpm durante 5 min. Luego la placa es lavada una vez en Lavado de Células Denley con 200 ul, seguido por un paso de aspiración a 100 ul de volumen final.

Para un ensayo no basado en células, cada pozo contiene una molécula fluorescente, tal como fluo-4. La proteína de fusión del invento es agregada al pozo, y se detecta un cambio de fluorescencia.

Para medir la fluorescencia de calcio intracelular, el FLIPR es colocado para los siguientes parámetros: (1) ganancia de sistema es 300-800 mW; (2) tiempo de exposición es 0.4 segundos; (3) F/stop de Camera es F/2; (4) Excitación es 488 nm; (5) Emisión es 530 nm; y (6) Adición de muestra es 50 ul. La emisión aumentada a 530 nm indica un evento de señalización extracelular producido por una proteína de fusión de albúmina del presente invento o una molécula inducida por una proteína de fusión de albúmina del presente invento, el cual ha resultado en un aumento en la concentración Ca++ intracelular.

EJEMPLO 31: Ensayo para Identificar Actividad de la Tirosinquinasa

Las protein-tirosinquinasas (PTK) representan un grupo diverso de quinasas de transmembrana y citoplásmicas. Dentro del grupo de protein-tirosinquinasas receptoras (RPTK) están los receptores para un rango de factores de crecimiento mitogénico y metabólico incluyendo PDGF, FGF, EGF, NGF, HGF, y subfamilias de receptores de insulina. Además existe una gran familia de RPTKs para la cual el correspondiente ligando es desconocido. Los ligandos para RPTKs incluyen principalmente proteinas pequeñas secretadas, pero también proteinas de unión de membrana y de matriz extracelular.

La activación de RPTK mediante ligandos involucra dimerización de receptores mediada por ligandos, resultando en transfosforilación de las subunidades receptoras y activación de las tirosinquinasas citoplásmicas. Las tirosinquinasas citoplásmicas incluyen tirosinquinasas asociadas a receptores de la familia-src (ej., src, yes, Ick, lyn, fyn) y protein-tirosinquinasas ligadas a no receptores y citosólicas, tal como la familia Jak, miembros de las cuales median median la transducción de señales activada por la superfamilia de citoquinas de receptores (ej., las Interleucinas, Interferones, GM-CSF, y Leptina).

Debido al amplio rango de factores conocidos capaces de estimular actividad de tirosinquinasa, es de interés identificar si una proteína de fusión de albúmina del presente invento o una molécula inducida por una proteína de fusión del presente invento es capaz de activar las rutas de transducción de señales de tirosinquinasa. Por lo tanto, el siguiente protocolo es diseñado para identificar tales moléculas capaces de activar las rutas de transducción de señales de tirosinquinasa.

Sembrar células objetivo (ej., queratinocitos primarios) a una densidad de aproximadamente 25.000 células por pozo en placas de 96 pozos Loprodyne Silent Screen compradas en Nalge Nunc (Naperville, IL). Las placas son esterilizadas con dos enjuagues de 30 minutos con etanol al 100%, enjuagadas con agua y secadas durante la noche. Algunas placas son recubiertas durante 2 horas con 100 ml de colágeno tipo I de calidad de cultivo célular (50 mg/ml), gelatina (2%) o polilisina (50 mg/ml), todas estas compradas en Sigma Chemicals (St. Louis, MO) o 10% Matrigel comprado en Becton Dickinson (Bedford, MA) o suero de ternero, enjuagado con PBS y almacenado a 4°C. El crecimiento de células en estas placas es probado al sembrar 5.000 células/pozo en medio de crecimiento y cuantificación indirecta del número de células a través del uso de alamarAzul como se describió por el fabricante Alamar Biosciences, Inc. (Sacramento, Ca) después de 48 horas. Cubiertas de placas Falcon #3071 de Becton Dickinson (Bedford, MA) son utilizadas para cubrir las Placas Loprodyne Silent Screen. Las placas de cultivos de células Falcon Microtest III también se pueden utilizar en algunos experimentos de proliferación.

Para preparar extractos, las células A431 son sembradas sobre membranas de nailon de placas de Loprodyne (20.000/200ml/pozo) y cultivadas durante la noche en medio completo. Las células están en reposo mediante incubación en medio basal libre de suero durante 24 horas. Después de tratamiento de 5-20 minutos con EGF (60 ng/ml) o una concentración diferente de una proteína de fusión de albúmina del invento, el medio fue retirado y 100 ml de amortiguador de extracción ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na3VO4, 2 mM Na4P2O7 y un coctel de inhibidores de proteasa (# 1836170) obtenido en Boeheringer Mannheim (Indianapolis, IN)) es agregado a cada pozo y la placa es agitada sobre un agitador rotatorio durante 5 minutos a 4°C. Luego la placa es colocada en un colector de transferencia de vacío y el extracto es filtrado a través de los fondos de membrana de 0.45 mm de cada pozo utilizando bomba de vacío. Los extractos son recolectados en una placa de captura/ensayo de 96 pozos en el fondo del colector de vacío y colocados inmediatamente sobre hielo. Para obtener extractos clarificados por centrifugación, el contenido de cada pozo, después de solubilización detergente durante 5 minutos, es retirado y centrifugado durante 15 minutos a 4 °C a 16.000 x g.

Probar los extractos filtrados para niveles de actividad de tirosinquinasa. Aunque muchos métodos para detectar actividad de tirosinquinasa son conocidos, en método es descrito en la presente.

Generalmente, la actividad tirosinquinasa de una proteína de fusión de albúmina del invento es evaluada al determinar su capacidad para fosforilar un residuo de tirosina sobre un

sustrato específico (un peptido biotinilado). Los péptidos biotinilados que se pueden utilizar para este propósito incluyen PSK1 (correspondiente a aminoácidos 6-20 de la quinasa de división celular cdc-p-34) y PSK2 (correspondiente a aminoácidos 1-17 de gastrina). Ambos péptidos son sustratos para un rango de tirosinquinasas y están disponibles en Boehringer Mannheim.

La reacción de tirosinquinasa es configurada al agregar los siguientes componentes en orden. Primero, agregar 10 ul de 5uM Peptido Biotinilado, luego 10ul de ATP/Mg2+(5mM ATP/50mM MgCl2), luego 10 ul de Amortiguador de Ensayo 5x (40 mM hidrocloruro de imidazol, pH 7.3, 40 mM beta-glicerofosfato, 1mM EGTA, 100mM MgCl2, 5 mM MnCl2, 0.5 mg/ml BSA), luego 5ul de Vanadato de Sodio (1mM), y luego 5ul de agua. Mezclar los componentes suavemente y preincubar la mezcla de reacción a 30 grados C duante 2 min. Iniciar la reacción al agregar 10ul de enzima de control o el sobrenadante filtrado.

Luego la reacción de ensayo de tirosinquinasa es terminada al agregar 10 ul de 120 mm EDTA y colocar las reacciones en hielo.

La actividad de tirosinquinasa es determinada al transferir 50 ul de mezcla de reacción a un modulo de placa de microtitulos (MTP) e incubar a 37 grados C durante 20 min. Esto permite a la placa de 96 pozos recubierta con estreptavidina asociarse con el péptido biotinilado. Lavar el módulo MTP con 300 ul/pozo de PBS cuatro veces. Luego agregar 75 ul de anticuerpo anti-fosfotirosina conjugado a peroxidasa de rábano (anti-P-tir-PDO(0.5 u/ml) a cada pozo e incubar a 37 grados C durante 1 hora. Lavar el pozo como antes.

Luego agregar 100 ul de solución de sustrato de peroxidasa (Boehringer Mannheim) e incubar a temperatura ambiente durante al menos 5 min (hasta 30 min). Medir la absorbancia de la muestra a 405 nm mediante el uso de un lector ELISA. El nivel de actividad de peroxidasa unida es cuantificado utilizando un lector ELISA y refleja el nivel de actividad de tirosinquinasa.

EJEMPLO 32: Ensayo para Identificar Actividad de Fosforilación.

Como un alternativa potencial y/o complemento al ensayo de actividad de protein-tirosinquinasa descrito en el Ejemplo 31, también se puede utilizar un ensayo que detecta activación (fosforilación) de principales intermedios de transducción de señales. Por ejemplo, como se describe a continuación un ensayo particular puede detectar fosforilación de tirosina de las quinasas Erk-1 y Erk-2. Sin embargo, la fosforilazión de otras moleculas, tales como Raf, JNK, p38 MAP, quinasa Map (MEK), quinasa MEK, Src, quinasa específica muscular (MuSK), IRAK, Tec, y Janus, así como cualquier otra molécula de fosfoserina, fosfotirosina, o fosfotreonina, se puede detectar al sustituir estas moléculas por Erk-1 o Erk-2 en el siguiente ensayo.

Especificamente, las placas de ensayos son hechas al recubrir los pozos de una placa ELISA de 96 pozos con 0.1 ml de proteína G (1 ul/ml) durante 2 horas a temperatura ambiente, (RT). Luego las placas son enjuagadas con PBS y bloqueadas con 3% BSA/PBS durante 1 h a temperatura ambiente. Luego las placas de proteína G son tratadas con 2 anticuerpos monoclonales comerciales (100 ng/pozo) contra Erk-1 y Erk-2 (1h a temperatura ambiente). Luego las placas de proteína G son tratadas con 2 anticuerpos monoclonales comerciales (100 ng/pozo) contra Erk-1 y Erk-2 (1 h a RT) (Santa Cruz Biotechnology). (para detectar otras moleculas, este paso se puede modificar facilmente al sustituir un anticuerpo monoclonal detectando cualquiera de las moleculas aneriormente descritas.) Después de 3-5 enjuagues con PBS, las placas son almacenadas a 4 °C hasta utilizar.

Células A431 son sembradas a 20.000/pozo en una placa de filtro Loprodyne de 96 pozos y cultivada durante la noche en medio de crecimiento. Luego las células son cosechadas durante 48 horas en medio basal (DMEM) y luego tratadas con EGF (6ng/pozo) o concentraciones

variables de la proteína de fusión del invento durante 5-20 minutos. Luego las células son solubilizadas y los extractos filtrados directamente en la placa de ensayo.

Después de incubación con el extracto durante 1 hora a temperatura ambiente, los pozos son nuevamente enjuagados. Como un control positivo, una preparación comercial de quinasa MAP (10 ng/pozo) se utiliza en cambio del extracto A431. Luego las placas son tratadas con un anticuerpo policlonal comercial (conejo) (1 ug/ml) el cual reconoce específicamente el epítope fosforilado de las quinasas Erk-1 y Erk-2 (1 hora a Temperatura Ambiente). El anticuerpo es biotinilado mediante procedimientos estándares. El anticuerpo policlonal unido luego es cuantificado mediante incubaciones sucesivas con agente mejorador de fluorescencia de Europio y Europio-estreptavidina en el instrumento DELFIA Wallac (fluorescencia resulta por tiempo). Una señal fluorescente aumentada sobre el fondo indica una fosforilación mediante la proteína de fusión del presente invento o una molecula inducida por una proteína de fusión de albúmina del presente invento.

EJEMPLO 33: Ensayo de Fosforilación.

Con el objeto de probar la actividad de fosforilación de una proteína de fusión de albúmina del invento, se utiliza un ensayo de fosforilación como se describe en la Patente Americana 5.958.405 (la cual se incorpora en la presente como referencia). Brevemente, la actividad de fosforilación se puede medir mediante fosforilación de un sustrato de proteína utilizando 32P-ATP gama rotulado y cuantificación de la radioactividad incorporada utilizando un contador de isotopos gama. La proteína de fusión del invento es incubada con el sustrato de proteína, 32P-ATP, y un amortiguador de quinasa. Luego el 32P incorporado en el sustrato es separado de 32P-ATP libre mediante electroforesis, y el 32P incorporado es contado y comparado con un control negativo. Los totales de radioactividad por encima del control negativo son indicativos de actividad de fosforilación de la proteína de fusión.

EJEMPLO 34: Detección de Actividad de Fosforilación (Activación) de una Proteína de Fusión de Albúmina del Invento en Presencia de Ligandos Polipéptidos.

Los métodos conocidos en el estado de la técnica o descritos en la presente se pueden utilizar para determinar la actividad de fosforilación de una proteína de fusión de albúmina del invento. Un método preferido para determinar actividad de fosforilación es mediante el uso del ensayo de fosforilación de tirosina como se describió en la Patente Americana 5.817.471 (incorporada en la presente como referencia).

EJEMPLO 35: Ensayo para la Estimulación de Células CD34+ de Médula Osea.

Este ensayo se basa en la capacidad de CD34+ humano para proliferar en presencia de factores de crecimiento hematopoyeticos y evalua la capacidad de proteínas de fusión del invento para estimular proliferación de células CD34+.

Se ha mostrado anteriormente que la mayoría de precursores maduros responden solamente a una única señal. Más precursores inmaduros requieren al menos dos señales para responder. Por lo tanto, para probar el efecto de proteínas de fusión del invento sobre actividad hematopoyetica de un amplio rango de células progenitoras, el ensayo contiene una proteína de fusión dada del invento en presencia o ausencia de factores de crecimiento hematopoyetico. Las células aisladas son cultivadas durante 5 días en la presencia de Factor de Células madre (SCF) en combinación con la muestra probada. SCF solo tiene un efecto muy limitado sobre la proliferación de células de médula ósea (BM), actuando en tales condiciones solamente como un factor de "superviviencia". Sin embargo, combinado con cualquier factor que exhibe efecto estimulatorio sobre estas células (ej., IL-3), SCF producirá un efecto sinergistico. Por lo tanto, si la proteína de fusión probada tiene un efecto estimulador sobre progenitores hematopoyeticos, tal actividad puede ser facílmente detectada. Puesto que las células BM normales tienen un bajo

nivel de células cíclicas, es probable que cualquier efecto inhibidor de una proteína de fusión dada no puede ser detectado. Por consiguiente, los ensayos de un efecto inhibitorio sobre progenitores es preferiblemente probado en células que son primero sometidas a estimulación *in vitro* con SCF+IL+3, y luego son colocadas en contacto con el compuesto que está siendo evaluado para inhibición de tal proliferación inducida.

Brevemente, las células CD34+ son aisladas utilizando métodos conocidos en el estado de la técnica. Las células que son licuadas y suspendidas en medio (medio libre de suero QBSF 60 con 1% L-glutamina (500 ml) Quality Biological, Inc. Gaithersburg, MD Cat# 160-204-101). Después de varios pasos de centrifugación suave a 200 x g, las células son dejadas reposar durante 1 hora. El conteo celular es ajustado a 2.5 x 10 5 células/ml. Durante este tiempo, se agrega 100 ul de agua esteril a los pozos periféricos de una placa de 96 pozos. La citoquina que se puede probar con una proteína de fusión de albúmina del invento en este ensayo es rhSCF (R&D Systems, Minneapolis, MN, Cat# 255-SC) a 50 ng/ml sola o en combinación con rhSCF y rhIL-3 (R&D Systems, Minneapolis, MN, Cat# 203-ML) a 30 ng/ml. Después de una hora, 10 ul de citoquinas preparadas, concentraciones variables de una proteína de fusión de albúmina del invento, y 20 ul de células diluidas son agregados a los medios ya presentes en los pozos para permitir un volumen total final de 100 ul. Luego las placas son colocadas en un incubador de CO2 al 5% a 37°C durante cinco días.

Diezocho horas antes de cosechar el ensayo, se agrega 0.5 uCi/pozo de [3H] Timidina en un volumen de 10 ul a cada pozo para determinar la tasa de proliferación. El experimento es terminado al cosechar las células de cada placa de 96 pozos hacia un filtermat utilizando el Cosechador Tomtec 96. Después de cosechar, los filtermats son secados, triturados y colocados en ensables OmniFilter conteniendo una placa OmniFilter y una bandeja OMniFilter. Se agrega 60 ul de Microscint a cada pozo y la placa es sellada con película selladora a presión TopSeal-A. Una etiqueta de código de barras 15 es fijada a la primera placa para conteo. Luego las placas selladas son cargadas y el nivel de radioactividad se determina a través de Packard Top Count y los datos impresos se recolectan para análisis. El nivel de radioactividad refleja la cantidad de proliferación celular.

Los estudios descritos en este ejemplo prueban la actividad de una proteína de fusión dada para estimular proliferación de células CD34+ de médula ósea. Alguien con experiencia en el estado de la técnica podría modificar facilmente los estudios de ejemplo para probar la actividad de proteínas de fusión y polinucleotidos del invento (ej., terapia genética) así como también agonistas y antagonistas de los mismos. La capacidad de una proteína de fusión de albúmina del invento para estimular la proliferación de células CD34+ de médula ósea indica que la proteína de fusión de albúmina y/o polinucleotidos que corresponden a la proteína de fusión son útiles para la diagnosis y tratamiento de trastornos que afectan el sistema inmune y hematopoiesis. Usos representativos se describen en la "Actividad Inmune" y secciones anterios de "Enfermedad Infecciosa", y en otra parte en la presente.

EJEMPLO 36: Ensayo para Respuesta Celular Aumentada de Matriz Extracelular (EMECR).

El objetivo del Ensayo de Respuesta Celular Aumentada de Matriz Extracelular es evaluar la capacidad de las proteínas de fusión del invento para actuar sobre células madre hematopoyeticas en el contexto de la señal inducida de matriz extracelular (ECM).

Las células responden a factores regulatorios en el contexto de señales recibidas desde el microambiente circundante. Por ejemplo, los fibroblastos, y células madre endoteliales y epiteliales fallan para replicarse en la ausencia de señales desde el ECM. Las células madre hematopoyéticas pueden experimentar auto-renovación en la médula ósea, pero no en cultivo de

suspensión *in vitro*. La capacidad de las células madre para experimentar autorenovación *in vitro* depende de su interacción con las células estromales y la fibronectina de proteínas ECM (fn). La adhesión de células a fn es mediada por los receptores de integrina alfa5Beta1 y alfa4Beta1, los cuales son expresados por células madre hematopoyeticas humanas y de ratón. Los factores que se integran con el ambiente ECM y son responsables de estimular auto-renovación de células madre aún no han sido identificados. El descubrimiento de tales factores es de gran interés en terapia genética y aplicaciones de transplantes de médula ósea.

Brevemente, las placas de 96 pozos tratadas con cultivo sin tejidos de poliestireno son recubiertas con fragmento fn a una concentración de revestimiento de 0.2 ug/cm2. Células de medula ósea de ratón son sembradas en placas (1000 células/pozo) en 0.2 ml de medio libre de suero. Las células cultivadas en presencia de IL-3 (5 ng/ml) + SCF (50 ng/ml) servirían como el control positivo, se esperan condiciones bajo las cuales ocurre poca auto-renovación pero diferenciación pronunciada de las células madre. Las proteínas de fusión de albúmina del invento son probadas con controles negativos apropiados en presencia y ausencia de SCF (5.0 ng/ml), donde el volumen de la composición administrada que contiene la proteína de fusión de albúmina del invento representa 10% del volumen de ensayo total. Luego las células sembradas son dejadas crecer en un incubador de cultivo de tejidos de ambiente bajo en oxígeno (CO2 al 5%, O2 al 7%, y N2 al 88%) durante 7 días. Luego el número de células de proliferación dentro de los pozos es cuantificado al medir incorporación de timidina en el ADN celular. La verificación de los hits positivos en el ensayo requerirá de caracterización fenotípica de las células, la cual se puede realizar al aumentar progresivamente el sistema de cultivo y al utilizar reactivos de anticuerpos apropiados contra antígenos de superficie célular y FACScan.

Si se encuentra que una proteína de fusión particular del presente invento es un estimulador de progenitores hematopoyeticos, la proteína de fusión y polinucleotidos correspondientes a la proteína de fusión pueden ser útiles por ejemplo, en la diagnosis y tratamiento de trastornos que afectan el sistema inmune y hematopoyesis. Usos representativos son descritos en las secciones "Actividad Inmune" y "Enfermedad Infecciosa" anteriores, y en cualquier parte en la presente. La proteína de fusión también puede ser útil en la expansión de células madre y progenitores comprometidos de varios linajes sanguineos, y en la diferenciación y/o proliferación de varios tipos de células.

Adicionalmente, la proteínas de fusión de albúmina del invento y polinucleotidos que codifican proteínas de fusión de albúmina del invento, también se pueden emplear para inhibir la proliferación y diferenciación de células hematopoyéticas y por lo tanto se pueden emplear para proteger células madre de médula ósea de agentes quimioterapéuticos durante quimioterapia. Este efecto antiproliferativo puede permitir administración de altas dosis de agentes quimioterapéuticos y, por lo tanto, tratamiento quimioterapéutico más efectivo.

Además, las proteínas de fusión del invento y polinucleotidos que codifican proteínas de fusión de albúmina del invento también pueden ser útiles para el tratamiento y diagnosis de trastornos hematopoyéticos relacionados tales como, anemia, pancitopenia, leucopenia, trombocitopenia o leucemia, puesto que las células estromales son importantes en la producción de células de linajes hematopoyéticos. Los usos incluyen cultivo *ex vivo* de células de médula ósea, transplante de médula ósea, reconstitución de médula ósea, radioterapia o quimioterapia de neoplasia.

EJEMPLO 37: Proliferación de Fibroblastos Dermales Humanos y Células de Musculo Liso Aórtico.

Una proteína de fusión de albúmina del invento es agregada a cultivos de fibroblastos dermales humanos normales (NHDF) y células del músculo liso aórtico humano (AoSMC) y dos

co-ensayos son realizados con cada maestra. El primer ensayo examina el efecto de la proteína de fusión sobre la proliferación de fibroblastos dermales humanos normales (NHDF) o células de músculo liso aórtico (AoSMC). El crecimiento aberrante de fibroblastos o células de músculo liso es una parte de varios procesos patológicos, incluyendo fibrosis y reestenosis. El segundo ensayo examina la producción de IL6 mediante NHDF y SMC. La producción de IL6 es una indicación de activación funcional. Las células activadas tendrán una producción aumentada de un número de citoquinas y otros factores, que pueden producir un resultado proinflamatorio o inmunomodulador. Los ensayos son realizados con y sin estimulación de co-TNFa, con el propósito de revisar la actividad coestimulatoria o inhibitoria.

Brevemente, el día 1, las placas negras de 96 pozos son configuradas con 1000 células/pozo (NHDF) o 2000 células/pozo (AoSMC) en 100 ul de medio de cultivo. El medio de cultivo NHDF contiene: medio basal Clonetics FB, 1 mg/ml de hFGF, 5mg/ml de insulina, 50mg/ml de gentamicina, FBS al 2%, mientras el medio de cultivo AoSMC contiene medio basal Clonetics SM, 0.5 ug/ml de hEGF, 5mg/ml de insulina, 1 ug/ml de hFGF, 50 mg/ml de gentamicina, 50 ug/ml anfotericina B, 5% FBS. Después de incubación a 37°C durante al menos 4-5 horas el medio cultivo es aspirado y reemplazado con medio de detención de crecimiento. El medio de detención de crecimiento para NHDF contiene medio basal de fibroblastos, 50mg/ml de gentamicina, 2% FBS, mientras el medio de detención de crecimiento para AoSMC contiene medio basal SM, 50mg/ml de gentamicina, 50ug/ml Anfotericina B, 0.4% FBS. Incubar a 37°C hasta el día 2.

El día 2, diluciones seriales y modelos de una proteína de fusión de albúmina del invento son diseñados tal que incluyan siempre controles de medio y controles de proteínas conocidas. Para experimentos de estimulación y de inhibición, las proteínas son diluidas en medio de detención de crecimiento. Para experimentos de inhibición, TNFa se agrega a una concentración final de 2ng/ml (NHDF) o 5ng/ml (AoSMC). Agregar medios de 1/3 vol conteniendo controles o una proteína de fusión de albúmina del invento e incubar a 37°C /CO2 al 5% hasta el día 5.

Transferir 60 ul desde cada pozo a otra placa de 96 pozos rotulada, cubrir con un sellador de placa, y almacenar a 4°C hasta el día 6 (para IL6 ELISA). A los restantes 100 ul en la placa de cultivo celular, agregar asépticamente Alamar Blue en una cantidad igual a 10% del volumen de cultivo (10ul). Retornar las placas al incubador durante tres a cuatro horas. Luego medir fluorescencia con excitación a 530nm y emisión a 590nm utilizando el CytoFluor. Esto proporciona los datos de estimulación/inhibición de crecimiento.

El día 5, se realiza el IL6 ELISA recubriendo una placa de 96 pozos con 50-100 ul/pozo de anticuerpo monoclonal IL6 antihumano diluido en PBS, pH 7.4, incubar a temperatura ambiente.

El día 6, vaciar las placas en el sumidero y secar sobre toallas de papel. Preparar amortiguador de ensayo conteniendo PBS con BSA al 4%. Bloquear las placas con 200 ul/pozo de amortiguador bloqueador Pierce Super Block en PBS durante 1-2 hr y luego lavar las placas con amortiguador de lavado (PBS, 0.05% Tween-20). Secar las placas sobre toallas de papel. Luego agregar 50 ul/pozo de anticuerpo Biotina-rotulado monoclonal IL6 antihumano diluido a 0.50 mg/ml. Hacer diluciones de IL-6 concentrada en medios (30, 10, 3, 1, 0.3, 0 ng/ml). Agregar muestras duplicadas a la fila superior de la placa. Cubrir las placas e incubar durante dos horas a temperatura ambiente sobre agitador.

Las placas son lavadas con amortiguador de lavado y secadas sobre toallas de papel. Diluir Estreptavidina EU-rotulada 1:1000 en amortiguador de ensayo, y agregar 100 ul/pozo. Cubrir la placa e incubar 1h a temperatura ambiente. Las placas son lavadas nuevamente con amortiguador de lavado y secadas sobre toallas de papel.

Agregar 100 ul/pozo de Solución de Aumento. Agitar durante 5 minutos. Leer la placa sobre el fluorometro DELFIA Wallac. Las lecturas de muestras triplicadas en cada ensayo fueron tabuladas y promediadas.

Un resultado positivo en este ensayo indica proliferación celular AoSMC y que la proteína de fusión de albúmina puede estar involucrada en proliferación de fibroblastos dermales y/o proliferación de células de músculo liso. Un resultado positivo también indica muchos usos potenciales de la proteína de fusión y polinucleotidos que codifican la proteína fusión de albúmina. Por ejemplo, respuestas inmunes y de inflamación, curación de heridas, y angiogénesis, como se detalla a través de esta especificación. Particularmente, las proteínas de fusión se pueden utilizar en curación de heridas y regeneración dermal, así como también en la promoción de vasculogenesis, tanto de los vasos sanguíneos como linfáticos. El crecimiento de vasos se puede utilizar en el tratamiento de, por ejemplo, enfermedades cardiovasculares. Adicionalmente, las proteínas de fusión que muestran actividad antagonística en este ensayo pueden ser útiles en el tratamiento de enfermedades, trastornos, y/o condiciones que involucran angiogénesis al actuar como un agente anti-vascular (ejemplo, anti- angiogénesis). Estas enfermedades, trastornos, y/o condiciones son conocidas en el estado de la técnica y/o son descritas en la presente, tal como, por ejemplo, malignidades, tumores sólidos, tumores benignos, por ejemplo, hemangiomas, neuromas acústicos, neurofibromas, tracomas, y granulomas piogénicos; placas arterosclerόticas; enfermedades angiogénicas oculares, por ejemplo, retinopatía diabética, retinopatía por premadurez, degeneración macular, rechazo de injerto corneal, glaucoma neovascular, fibroplasia retrolental, rebeosis, retinoblastoma, uvietis y pterigio (crecimiento de vasos sanguineos anormal) del ojo; artritis reumatoide, psoriasis; curación retardada de heridas; endometriosis; vasculogénesis, granulaciones; cicatrices hipertróficas, (queloides); fracturas en no uniones; esclerοderma; tracoma, adhesiones vasculares, angiogénesis del miocardio; colaterales coronarios, colaterales cerebrales, malformaciones arteriovenosas, angiogénesis de extremidades isquémicas, síndrome de Osler-Webber; neovascularización de placas; telangestasia; articulaciones hemofilicas; angiofibroma; displasia fibromuscular, granulación de heridas, enfermedad de Crohn; y ateroesclerosis.

EJEMPLO 38: Expresión de Molécula de Adhesión Celular (CAM) sobre Células Endoteliales.

El reclutamiento de linfocitos hacia áreas de inflamación y angiogénesis involucra interacciones específicas de receptores-ligandos entre moléculas de adhesión de superficie celular (CAMs) sobre linfocitos y el endotelio vascular. El proceso de adhesión, tanto en configuraciones normales como patológicas, sigue una cascada de múltiples etapas que involucra expresión de moleculas-1 de adhesión intercelular (CAMP-1), moléculas-1 de adhesión de células vasculares (VCAM-1), moleculas-1 de adhesión de leucocitos endoteliales (E-selectina) sobre células endoteliales (EC). La expresión de estas moléculas y otras sobre el endotelio vascular determina la eficiencia con la cual los leucocitos pueden adherirse a la vasculatura local y extravasarse hacia el tejido local durante el desarrollo de una respuesta inflamatoria. La concentración local de citoquinas y factor de crecimiento participan en la modulación de la expresión de estas CAMs.

Brevemente, las células endoteliales de vena umbilical humana (HUVECs) son cultivadas en una placa de 96 pozos estándar hasta confluencia, el medio de crecimiento de retirado de las células y reemplazado con 100 ul de Medio 199 (FBS al 10%). Muestras para prueba (conteniendo una proteína de fusión de albúmina del invento) y controles positivos o negativos son agregados a la placa en triplicado (en volumenes de 10 ul). Luego las placas son incubadas a 37 grados C durante ya sea 5 h (expresión de selectina e integrina) o 24 h (expresión de integrina solamente). Las placas son aspiradas para remover medio y 100 ul de 0.1%

paraformaldehido-PBS (con Ca++ y Mg++) se agrega a cada pozo. Las placas son mantenidas a 4°C durante 30 min. El fijador es retirado de los pozos y los pozos son lavados IX con PBS(+Ca,Mg)+0.5% BSA y drenados. Se Agrega 10 ul de anticuerpo primario diluido a los pozos de prueba y control. Anti-IVAM-1-Biotina, Anti-VCAM-1-Biotina y Anti-E-selectina-Biotina se utilizan a una concentración de 10ug/ml (dilución 1:10 de 0.1 mg/ml anticuerpo concentrado). Las células son incubadas a 37°C durante 30 minutos en un ambiente humedecido. Los pozos son lavados X3 con PBS(+Ca, Mg)+0.5%BSA. Una tableta de Fosfato p-Nitrofenol pNPP es disuelta en 5 ml de amortiguador de glicina (pH 10.4). 100 ul de sustrato de pNPP en amortiguador de glicina es agregado a cada pozo de prueba. Pozos estándares en triplicado son preparados a partir de la dilución de trabajo de ExtrAvidina- Fosfatosa Alcalina en amortiguador de glicina: 1:5000 (100) > 10 -0.5 > 10-t > 10-1.5. 5 ul de cada dilución son agregados a pozos triplicados y el contenido AP resultante en cada pozo es 5.50 ng, 1.74 ng,g 0.55 ng, 0.18 ng. Luego se agregan 100 ul de reactivo pNNP a cada uno de los pozos estándares. La placa es incubada a 37°c durante 4h. Un volumen de 50 ul de 3M NaOH es agregado a todos los pozos. La placa es leida sobre un lector de placa a 405 nm utilizando la opción de resta de fondo se utiliza sobre pozos vacíos llenados con amortiguador de glicina solamente. Adicionalmente, el modelo es configurado par indicar la concentración de conjugado AP en cada pozo estándar [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Los resultados son indicados como cantidad de conjugado AP unido en cada muestra.

EJEMPLO 39: Ensayo de Proliferación de Células Endoteliales Alamar Blue.

Este ensayo se puede utilizar para determinar cuantitativamente inhibición mediada por proteínas de proliferación bFGF-inducida de células endoteliales linfáticas bobinas (LECs), células endoteliales aórticas bobinas (BAECs) o células miometriales uterinas microvasculares humanas (UTMECs). Este ensayo incorpora un indicador de crecimiento fluorométrico basado en detección de actividad metabólica. Un ensayo de Proliferación Alamar Azul estándar es preparado en EGM-2MV con 10 ng/ml de bFGF agreado como una fuente de estimulación de células endoteliales. Este ensayo se puede utilizar con una variedad de células endoteliales con cambios leves en medio de crecimiento y concentración celular. Las diluciones de lotes de proteínas a ser probadas son diluidas según sea apropiado. Se utiliza medio libre de suero (GIBCO SFM) sin bFGF como un control no estimulado y Angiostatina o TSP-1 se incluyen como controles inhibitorios conocidos.

Brevemente, LEC, BAECs o UTMECs son sembrados en medios de crecimiento a una densidad de 5000 a 2000 células/pozo en una placa de 96 pozos y se colocan a 37 grados C durante la noche. Después de incubación de las células, el medio de crecimiento es retirado y reemplazado con EC-SFM GIBCO. Las células son tratadas con las diluciones apropiadas de una proteína de fusión de albúmina del invento o muestra de proteínas de control (preparada en SFM) en pozos triplicados con bFGF adicional a una concentración de 10 mg/ml. Una vez las células han sido tratadas con las muestras, las placas son colocadas devuelta en el incubador a 37°C durante tres días. Después de 3 días se agrega 10 ml de azul de alamar concentrado (Biosource Cat# DAL1100) a cada pozo y las placas son colocadas devuelta en el incubador a 37°C durante cuatro horas. Luego las placas son leidas a excitación de 530 nm y emisión de 590 nm utilizando el lector de fluorescencia CytoFluor. La salida directa es registrada en unidades de fluorescencia relativa.

Alamar blue es un indicador de oxidación-reducción que fluorece y cambia de color en respuesta a reducción química de medio de crecimiento que resulta a partir de crecimiento celular. A medida que las células crecen en cultivo, la actividad metabólica innata resulta en una reducción química del ambiente circundante inmediato. La reducción relacionada con

crecimiento hace que el indicador cambie de forma oxidada (azul no fluorescente) a forma reducida (rojo fluorescente) (es decir, la proliferación estimulada producirá una señal más fuerte y la proliferación inhibida producirá una señal más debil y la señal total es proporcional al número total de células así como también a su actividad metabólica). El nivel de actividad de fondo es observado con el medio de inanición solo. Esto se compara a la salida observada desde las muestras de control positivo (bFGF en medio de crecimiento) y diluciones de proteínas.

EJEMPLO 40: Detección de Inhibición de una Reacción de Linfocitos Mixta.

Este ensayo se puede utilizar para detectar y evaluar inhibición de una Reacción de Linfocitos Mixta (MLR) mediante proteínas de fusión del invento. La inhibición de un MLR se puede deber a un efecto directo sobre proliferación celular y viabilidad, modulación de moleculas coestimuladoras sobre células de interacción, modulación de adhesividad entre linfocitos y células accesorias, o modulación de producción de citoquinas mediante células accesoarias. Múltiples células pueden estar dirigidas por las proteínas de fusión de albúmina que inhiben MLR puesto que la fracción mononuclear de sangre periférica utilizada en este ensayo incluye linfocitos T, B y asesinos naturales, así como también monocitos y células dendríticas.

Las proteínas de fusión de albúmina del invento que inhiben MLR pueden encontrar aplicación en enfermedades asociadas con activación o proliferación de linfocitos y monocitos. Estas incluyen, pero no están limitadas a, enfermedades tales como asma, artritis, diabetes, condiciones dérmicas inflamatorias, psoriasis, eczema, lupus sistémico eritematoso, esclerosis múltiple, glomerulonefritis, enfermedad intestinal inflamatoria, enfermedad de Crohn, colitis ulcerativa, arterioesclerosis, cirrosis, enfermedad de injerto vs huésped, enfermedad de huésped vs. injerto, hepatitis, leucemia y linfoma.

Brevemente, PBMCs de donantes humanos son purificados mediante centrifugación de gradiente de densidad utilizando Medio de Separación de Linfocitos (LSM, densidad 1.0770 g/ml, Organon Teknika Corporation, West Chester, PA). PBMCs de dos donantes son ajustados a 2 x 10 6 células/ml en RPMI-1640 (Life Technologies, Grand Island, NY) suplementado con FCS 10% y 2 mM glutamina. PBMCs de un tercer donante es ajustado a 2 x 10 5 células/ml. 50 ul de PBMCs de cada donante es agregado a pozos de una placa de microtitulos de fondo redondo de 96 pozos. Diluciones del material de prueba de proteína de fusión (50 ul) es agregado en triplicado a los pozos de microtitulos. Las muestras de prueba (de la proteína de interes) son agregadas para dilución final de 1:4; rhuIL-2 (R&D Systems, Minneapolis, Mn número de catalogo 202-IL) es agregada a una concentración final de 10 ug/ml. Las células son cultivadas durante 7-8 días a 37°C en CO2 al 5%, y 1 uC de [3H]timidina es agregada a los pozos durante las últimas 16 horas de cultivo. Las células son cosechadas y la incorporación de timidina determinada utilizando un Packard TopCount. Los datos son expresados como la media y desviación estándar de determinaciones triplicadas.

Las muestras de la proteína de fusión de interés son seleccionadas en experimentos separados y comparadas con el tratamiento de control negativo, anti-CD4 mAb, el cual inhibe proliferación de linfocitos y el tratamiento de control positivo, IL-2 (ya sea como material recombinante o sobrenadante), el cual aumenta la proliferación de linfocitos.

EJEMPLO 41: Ensayos para Actividad de Proteasa.

El siguiente ensayo se puede utilizar para evaluar actividad de proteasa de una proteína de fusión de albúmina del invento.

Cimografia de gelatina y caseina es realizada esencialmente como se describe (Heusen, *Anal. Biochem.*, 102:196-202 (1980); Wilson, *Journal of Urology*, 149:653-658 (1993)). Las muestras son ejecutadas sobre poliacriamida al 10%/ geles de SDS al 1% conteniendo orcaseina de gelatina al 1%, empapada en triton al 2.5% a temperatura ambiente durante 1 hora, y en 0.1 M

glicina, pH 8.3 a 37°C durante 5 a 16 horas. Después de teñido en áreas negras amido de proteolisis aparece como áreas claras contra el fondo azul-negro. Tripsina (Sigma T8642) se utiliza como un control positivo.

La actividad de proteasa también se determina al monitorear la excisión de ester etilico de n-a-benzoil-L-arginina (BAEE) Sigma B-4500. Las reacciones son configuradas en (25mM NaPO4, 1mM EDTA, y 1mM BAEE), pH 7.5. Las muestras son agregadas y el cambio en absorbancia a 260 nm es monitoreado sobre el espectrometro Beckman DU-6 en el modo accionado por tiempo. La tripsina se utiliza como un control positivo.

Ensayos adicionales basados en la liberación de péptidos solubles en ácido a partir de caseina o hemoglobina medida como absorbancia a 280 nm o colorimetricamente utilizando el método Folin son realizados como se describe en Bergmeyer, *Methods of Enzymatic Analysis*, 5 (1984). Otros ensayos involucran solubilización de sustratos cromogénicos (Ward, *Applied Science*, 251-317 (1983)).

EJEMPLO 42: Identificación de Especificidad de Sustrato de Proteasa de Serina.

Los métodos conocidos en el estado de la técnica o descritos en la presente se pueden utilizar para determinar la especificidad de sustrato de las proteínas de fusión de albúmina del presente invento que tienen actividad de proteasa de serina. Un método preferido para determinar especificidad de sustrato es mediante el uso de bibliotecas combinatoriales sintéticas de escaneo posicional como se describe en GB 2 324 529 (incorporada en la presente en su totalidad).

EJEMPLO 43: Ensayos de Unión de Ligandos.

El siguiente ensayo se puede utilizar para evaluar la actividad de unión de ligandos de una proteína de fusión de albúmina del invento.

Los ensayos de unión de ligandos proporcionan un método directo para indagar la farmacología de receptores y son adaptables a un formato de alto rendimiento. El ligando purificado para una proteína de fusión de albúmina del invento es radiorotulado a actividad específica alta (50-2000 Ci/mmol) para estudios de unión. Luego se determina que proceso de radiorotulación no disminuye la actividad del ligando hacia la proteína de fusión. Las condiciones de ensayo para amortiguadores, iones, pH y otros moduladores tales como nucleotidos son optimizadas para establecer una proporción de señal factible a ruido tanto para membrana como para fuentes polipeptídicas de células completas. Para estos ensayos, la unión polipéptida específica es definida como radioactividad total asociada menos la radioactividad medida en presencia de un exceso de ligando competitivo no rotulado. Donde sea posible, más de un ligando competitivo es utilizado para definir unión no específica residual.

EJEMPLO 44: Ensayo Funcional en Xenopus Oocitos

Los transcriptos de ARN tapados a partir de modelos plásmidos linearizados que codifican una proteína de fusión de albúmina del invento son sintetizados in vitro con polimerasas de ARN según procedimientos estándares. Los transcritos in vitro son suspendidos en agua y una concentración final de 0.2 mg/ml. Los lobulos ováricos son retirados de sapos hembra adultos, se obtienen oocitos desfoliculados Stage V, y los transcriptos de ARN (10 ng/oocito) son inyectados en un bolo de 50 nl utilizando un aparato de microinyección. Se utilizan dos pinzas de voltaje de electrodos para medir las corrientes a partir de *Xenopus oocitos* individuales en proteína de fusión de respuesta y exposición agonista polipéptida. Las lecturas son hechas en medio Barth libre de Ca2+ a temperatura ambiente. El sistema Xenopus se puede utilizar para seleccionar ligandos conocidos y extractos de tejidos/células para activar ligandos.

EJEMPLO 45: Ensayos microfisiométricos.

La activación de una amplia variedad de sistemas mensajeros secundarios resulta en extrusión de pequeñas cantidades de ácido desde una célula. El ácido formado es principalmente

el resultado de la actividad metabólica aumentada requerida para alimentar el proceso de señalización intercelular. Los cambios de pH en los medios que circundan la célula son muy pequeños pero detectables mediante el microfisiometro CYTOSENSOR (Molecular Devices Ltd., Menlo Park, Calif.). Por lo tanto EL CYTOSENSOR es capaz de detectar la capacidad de una proteína de fusión de albúmina del invento para activar mensajeros secundarios que están acoplados a una energía que utiliza ruta de señalización intracelular.

EJEMPLO 46: Selección de Sobrenadantes de Extractos/Células.

Existe un gran número de receptores mamíferos para los cuales ... (agonista). Por lo tanto, los ligandos activos para estos receptores no se pueden incluir dentro de los bancos de ligandos identificados hasta la fecha. Por consiguiente, las proteínas de fusión de albúmina del invento también se pueden clasificar funcionalmente (utilizando calcio, cAMP, microfisiometro, electrofisiología de oocitos, etc., clasificaciones funcionales) contra extractos de tejidos para identificar ligandos naturales para la porción de proteína terapéutica y/o porción de proteína de albúmina de una proteína de fusión de albúmina del invento. Los extractos que producen respuestas funcionales positivas se pueden subfraccionar secuencialmente hasta que un ligando activador es aislado e identificado.

EJEMPLO 47: Ensayo de Unión-ATP.

El siguiente ensayo se puede utilizar para evaluar actividad de unión ATP de proteínas de fusión del invento. La actividad de unión-ATP de una proteína de fusión de albúmina del invento se puede detectar utilizando el ensayo de unión descrito en la Patente U.S. 5.858.719, el cual se incorpora en la presente como referencia en su totalidad. Brevemente, la unión ATP a una proteína de fusión de albúmina del invento es medida a través de rotulación de fotoafinidad con 8-azido-ATP en un ensayo de competición. Las mezclas de reacción conteniendo 1 mg/ml de proteína de transporte ABC son incubadas con concentraciones variables de ATP, o el analogo ATP no hidrolizable adenil-5'-imidodifosfato durante 10 minutos a 4°C. Una mezcla de 8-azido-ATP (Sigma Chem Corp., St. Souis, MO.) más 8-azido-ATP (32P-ATP)(5 mCi/umol, ICN, Irvine CA) es agregada a una concentración final de 100 uM y aliquotas de 0.5 ml son colocadas en los pozos de una placa de porcelana sobre hielo. La placa es irradiada utilizando una lampara UV de 254 nm de onda corta a una distancia de 2.5 cm desde la placa para dos intervalos de un minuto con un intervalo de enfriamiento de un minuto entre estos. La reacción es detenida mediante la adición de una concentración final de de 2 mM. Las incubaciones son sometidas a electroforesis SDS-PAGE, secadas y autoradiograficadas. Las bandas de proteínas correspondientes a las proteínas de fusión de albúmina del invento son cortadas y la radioactividad es cuantificada. Una disminución en radioactividad con ATP o adenil-5'-amidodifosfato creciente suministra una medida de afinidad ATP a la proteína de fusión.

Ejemplo 48: Identificación de Proteínas de Transducción de Señales que Interactúan con una Proteína de Fusión de Albúmina del Presente Invento.

Las proteínas de fusión de albúmina del invento pueden servir como herramientas de investigación para la identificación, caracterización y purificación de proteínas de rutas de transducción de señales o proteínas receptoras. Brevemente, una proteína de fusión rotulada del invento es útil como un reactivo para la purificación de moléculas con las cuales esta interactúa. En una realización de purificación de afinidad, una proteína de fusión de albúmina del invento es acoplada de manera covalente a una columna de cromatografía. Extracto libre de células derivado de células objetivo putativas, tales como tejidos de carcinoma, es pasado sobre la columna, y las moléculas con afinidad apropiada se unen a la proteína de fusión de albúmina. El complejo de proteínas es recuperado desde la columna, disociado, y la molécula recuperada es sometida a secuenciamiento de proteínas N-terminal. Luego esta secuencia de aminoácidos es utilizada para

identificar la molécula capturada o para diseñar sondas oligonucleotidas degeneradas para clonar el gen relevante a partir de una biblioteca de cADN apropiada.

EJEMPLO 49:G Bioensayo IL-6.

Una variedad de ensayos son conocidos en el estado de la técnica para probar los efectos proliferativos de una proteína de fusión de albúmina del invento. Por ejemplo, uno de estos ensayos es el Bioensayo IL-6 tal como lo describe Marz (*Proc. Natl. Acad. Sci., USA, 95:*3251-56 (1998), el cual se incorpora en la presente como referencia). Después de 68 horas a 37°C, el número de células viables es medido al agregar azul de tiazolilo de sal de tetrazolio (MTT) e incubar durante 4 horas más a 37°C. Las células B9 son sometidas a lisis mediante SDS y la densidad óptica es medida a 570 nm. Los controles conteniendo IL-6 (positiva) y no citoquina (negativa) son Briefly, las células murinas B9 dependientes de IL-6 son lavadas tres veces en medio libre de IL-6 y sembradas en placas a una concentración de 5.000 células por pozo en 50 ul, y se agregan 50 ul de proteína de fusión del invento. La proliferación aumentada en la muestra de prueba (conteniendo una proteína de fusión de albúmina del invento) relativa al control negativo es indicativa de efectos proliferativos mediados por la proteína de fusión.

EJEMPLO 50: Soporte de Supervivencia de Neuronas de Embriones de Pollo.

Para probar si la viabilidad de células neuronales simpatéticas es soportada por una proteína de fusión de albúmina del invento, se puede utilizar el ensayo de supervivencia neuronal de embriones de pollo de Senaldi (*Proc. Natl. Acad. Sci., USA, 96:*11458-63 (1998), el cual se incorpora en la presente como referencia). Brevemente, las neuronas motoras y simpatéticas son aisladas de embriones de pollo, resuspendidas en medio L15 (con FCS al 10%, glucosa, selenita de sodio, progesterona, conalbúmina, putrescina, e insulina; Life Technologies, Rockville, MD.) y medio Eagles modificado de Dulbecco [con FCS al 10%, glutamina, penicilina, 25 mM amortiguador Hepes (pH 7.2); Life Technologies, Rockville, MD.], respectivamente, e incubaas a 37°C en CO2 al 5%, en presencia de concentraciones diferentres de proteína de fusión purificada del invento, así como también un control negativo que carece de cualquier citoquina. Después de 3 días, la superviviencia de neuronas es determinada por evaluación de morfología celular, y el través del uso del ensayo colorimétrico de Mosmann (Mosmann, T., *Immunol. Methods. 65:*55-63 (1983)). La viabilidad de células neuronales aumentada comparada con los controles que carecen de citoquinas es indicativa de la capacidad de la proteína de fusión de albúmina para aumentar la supervivencia de células neuronales.

EJEMPLO 51: Ensayo para Actividad de Fosfatasa.

El siguiente ensayo se puede utilizar para evaluar la actividad de serina/treonina fosfatasa (PTPasa) de una proteína de fusión de albúmina del invento.

Para probar la actividad de serina/treonina fosfatasa, se pueden utilizar ensayos ampliamente conocidos por aquellos con experiencia en el estado de la técnica. Por ejemplo, la actividad de serina/treonina fosfatasa de una proteína de fusión de albúmina del invento se puede medir utilizando un kit de ensayo PSPasa de New England Biolabs, Inc. La proteína básica de mielina (MyBP), un sustrato para PSPasa, es fosforilada para residuos de serina y treonina con protein-quinasa dependiente de cAMP en presencia de [32P]ATP. Luego la actividad de la proteína serina/treonina fosfatasa es determinada al medir la liberación de fosfatasa inorgánica a partir de MyBP rotulada por 32P.

EJEMPLO 52: Interacción de Serina/Treonina Fosfatasas con Otras Proteínas.

Las proteínas de fusión del invento con actividad de serina/treonina fosfatasa (ej., como se determinó en el ejemplo 51) son útiles, por ejemplo, como herramientas de investigación para la identificación, caracterización y purificación de proteínas de interacción adicional o proteínas receptoras, u otras proteínas de rutas de transducción de señales. Brevemente, una proteína de

fusión rotulada del invento es útil como un reactivo para purificación de moléculas con las cuales ésta interactúa. En una realización de purificación de afinidad, una proteína de fusión de albúmina del invento es acoplada covalentemente a una columna de cromatografía. Extracto libre de células derivado de células objetivo putativas, tales como células neuronales o de hígado, es pasado sobre la columna, y las moléculas con afinidad apropiada se unen a la proteína de fusión. El complejo de proteínas de fusión es recuperado desde la columna, disociado, y la molécula recuperada es sometida a secuenciamiento de proteínas N-terminal. Luego ésta secuencia de aminoácidos es utilizada para identificar la molécula capturada o para diseñar sondas oligonucleotidas degeneradas para clonar el gen relevante a partir de una biblioteca de cADN apropiada.

EJEMPLO 53: Ensayo para Actividad de Heparanasa.

Existen numerosos ensayos conocidos en el estado de la técnica que se pueden emplear para probar la actividad de heparanasa de una proteína de fusión de albúmina del invento. En un ejemplo, la actividad de heparanasa de una proteína de fusión de albúmina del invento, es probada como lo describe Voldavsky, (Vlodavsky, Nat. Med., 5:793-802 (1999)). Brevemente, los lisados celulares; los medios acondicionados, células intactas (1 x 10 6 células por disco de 35 mm), sobrenadante de cultivo celular, o proteína de fusión purificada son incubados durante 18 horas a 37°C, pH 6.2-6.6, con ECM rotulado con 35S o ECM soluble derivado de proteoglicanos pico I. El medio de incubación es centrifugado y el sobrenadante es analizado mediante filtración de gel sobre una columna de sefarosa CL-6B (0.9 x 30 cm). Las fracciones son eludidas con PBS y su radioactividad es medida. Los fragmentos de degradación de cadenas laterales de heparina son eluidos a partir de Sefarosa 6B a 0.5 < Kav <0.8 (pico II). Cada experimento es realizado al menos tres veces. Los fragmentos de degradación correspondientes al "pico II" como lo describió Vlodavsky, son indicativos de la actividad de una proteína de fusión de albúmina del invento para dividir sulfato de heparina.

EJEMPLO 54: Inmovilización de Biomoléculas

Este ejemplo proporciona un método para la estabilización de una proteína de fusión de albúmina del invento en constructos de bicapa lípida de células no huesped (ver, ej., Bieri, Nature Biotech 17:1105-1108 (1999), incorporado en la presente como referencia en su totalidad) el cual se puede adaptar para el estudio de proteínas de fusión de invento en varios ensayos funcionales descritos anteriormente. Brevemente, la química específica de carbohidratos para biotinilación se utiliza para confinar una etiqueta de biotina a una proteína de fusión de albúmina del invento, permitiendo así orientación uniforme ante inmovilización. Una solución de 50 uM de una proteína de fusión de albúmina del invento en membranas lavadas es incubada con 20 mM de NaIO4 y 1.5 mg/ml (4mM) BACH o 2 mg/ml (7.5 mM) biotin-hidrazida durante 1 hora a temperatura ambiente (volumen de reacción, 150 ul). Luego la muestra es dializada (Pierce Slidealizaer Cassett, 10 kDa corte; Pierce Chemical Co. Rockford IL) a 4C primero durante 5 h, intercambiando el amortiguador cada hora, y finalmente durante 12 horas contra 500 ml de amortiguador R (0.15 M NaCl, 1 mM MgCl2, 10 mM de fosfato de sodio, pH 7). Justo antes de la adición en una probeta, la muestra es diluida 1:5 en amortiguador ROG50 (Amortiguador R suplementado con 50 mM octilglucosida).

Ejemplo 55: Ensayos para Actividad de Metaloproteinasa.

Las metaloproteinasas son hidrolasas peptídicas las cuales utilizan iones metálicos, tal como Zn2+, como el mecanismo catalítico. La actividad de metaloproteinasa de una proteína fusión de albúmina del presente invento se puede probar según los métodos conocidos en el estado de la técnica. Se proporcionan los siguientes métodos de ejemplo:

Proteolisis de alfa-2-macroglobulina

Para confirmar la actividad de proteasa, una proteína de fusión purificada del invento es mezclada con el sustrato alfa-2-macroglobulina (0.2 unidades/ml; Boehringer Mannheim, Alemania) en amortiguador de ensayo iX (50 mM HEPES, pH 7.5, 0.2 M NaCl, 10 mM CaCl2, 25 uM ZnCl2 y 0.05% Brij-35) e incubada a 37°C durante 1-5 días. Se utiliza tripsina como control positivo. Los controles negativos contienen solamente alfa-2-macroglobulina en amortiguador de ensayo. Las muestras son recolectadas y bullidas en amortiguador de muestra SDS-PAGE conteniendo 2-mercaptoetanol al 5% durante 5 minutos, luego cargadas sobre 8% gel de SDS-poliacrilamina. Después de electroforesis de las proteínas son visualizadas mediante teñido de plata. La proteolisis es evidente por la aparición de bandas de bajo peso molecular en comparación con el control negativo.

Inhibición de proteolisis de alfa-2-macroglobulina mediante inhibidores de metaloproteinasas

Inhibidores de metaloproteinasa conocidos (quelantes metálicos (EDTA, EGTA, y HgCl2), inhibidores de metaloproteinasa péptidica (TIMP-1 y TIMP-2), e inhibidores MMP de moléculas pequeñas comerciales) también se pueden utilizar para caracterizar actividad proteolítica de una proteína de fusión de albúmina del invento. Tres inhibidores MMP sintéticos que se pueden utilizar son: inhibidor MMP I, [IC50 = 1.0 uM contra MMP-1 y MMP-8; IC50 = 30 uM contra MMP-9; IC50 = 150 uM contra MMP-3]; MMP-3 (estromelisina) inhibidor I [IC50 = 5 uM contra MMP-3], MMP-3 inhibidor II [Ki = 130 nM contra MMP-3]; inhibidores disponibles a través de Calbiochem, catalogo # 444250, 444218, y 444225, respectivamente). Brevemente, concentraciones diferentes de inhibidores MMP de moléculas pequeñas son mezclados con una proteína de fusión purificada del invento (50 ug/ml) en 22.9 ml de amortiguador HEPES 1x (50 mM HEPES, pH 7.5, 0.2 M NaCl, 10 mM CaCl2, 25 uM ZnCl2, y 0.05% Brij-35) e incubados a 37°C durante 20 horas. Las reacciones son detenidas al agregar amortiguador de muestra 4x y bullir inmediatamente durante 5 minutos. Después de SDS-PAGE, las bandas de proteínas son visualizadas mediante tinta de plata.

Ensayos de División de Sustratos de Péptidos Fluorogénicos Sintéticos

La especificidad de sustratos para proteínas de fusión del invento con actividad de metaloproteinasa demostrada se puede determinar utilizando técnicas conocidas en el estado de la técnica, tal como utilización de sustratos de péptidos fluorogénicos sintéticos (disponibles en BACHEM Bioscience Inc). Los sustratos de prueba incluyen M-1985, M-2225, M-2105, M-2110, y M-2225. Los primeros cuatro son sustratos MMP y el último es un sustrato de enzima de conversión (TACE) de factor-alfa de necrosis tumoral (TNF-alfa). Los sustratos preferiblemente son preparados en sulfoxido de dimetilo (DMSO) y agua. Las soluciones concentradas son 50-500 uM. Los ensayos fluorescentes son realizados mediante el uso de un espectrómetro de luminiscencia Perkin Elmer LS 50B equipado con un baño de agua a temperatura constante. La excitación gama es 328 nm y la emisión gama es 393 nm. Brevemente, el ensayo es realizado al incubar 176 ul de amortiguador HEPES 1x (0.2 M NaCl, 10 mM CaCl2, Brij-35 al 0.05% y 50 mM HEPES, pH 7.5) con 4 ul de solución de solución de sustrato (50 uM) a 25 °C durante 15 minutos, y luego agregando 20 ul de una proteína de fusión purificada del invento en la probeta de ensayo. La concentración final de sustrato es 1 uM. Las tasas de hidrolisis inicial son monitoreadas durante 30 min.

EJEMPLO 56: Ocurrencia de Diabetes en Ratones NOD.

Ratones hembra NOD (diabéticos no obesos) son caracterizados al visualizar IDDM con un curso similar a aquel encontrado en humanos, aunque la enfermedad es más pronunciada en ratones NOD hembra que en machos. De aquí en adelante, a menos que se afirme lo contrario, el término "ratón NOD" se refiere a un ratón NOD hembra. Los ratones NOD tienen una

destrucción progresiva de células beta la cual es causada por una enfermedad autoinmune crónica. Por lo tanto, los ratones NOD comienzan la vida con euglicemia, o niveles normales de glucosa sanguínea. Aproximadamente entre 15 y 16 semanas de edad, sin embargo, los ratones comienzan a volverse hiperglicémicos, indicando la destrucción de la mayoría de sus células beta pancreáticas y la correspondiente incapacidad del páncreas para producir insulina suficiente. Por lo tanto, tanto la causa como la progresión de la enfermedad son similares a pacientes humanos IDDM.

En ensayos *in vivo* la eficacia de los regímenes de inmunización se puede evaluar en ratones hembra NOD/LtJ (comercialmente disponibles en The Jackson Laboratory, Bar Harbor, Me). En la literatura, se reporta que 80% de los ratones hembras desarrollan diabetes a las 24 semanas de edad y el ataque de insulitis comienza entre 6-8 semanas de edad. Los ratones NOD endogámicos y altamente sensibles a una variedad de estrategias inmunoreguladoras. Ratones NOD aldultos (6-8 semanas de edad) tienen una masa promedio de 20-25 g.

Estos ratones pueden ser no tratados (control), tratados con terapéuticos del presente invento (ej., proteínas de fusión de albúmina del invento y fragmentos y variantes en las mismas), solos o en combinación con otros compuestos terapéuticos divulgados anteriormente. El efecto de estos diferentes tratamientos sobre la progresión de diabetes se puede medir como sigue:

A las 14 semanas de edad, los ratones NOD hembra se pueden fenotipificar según tolerancia a glucosa. La tolerancia a glucosa se puede medir con la prueba de tolerancia a glucosa intraperitoneal (IPGTT). Brevemente, se extrae sangre del plexo paraorbital entre 0 y 60 minutos después de la inyección intraperitoneal de glucosa (1 g/kg de peso corporal). La tolerancia normal es definida como glucosa de plasma a 0 minutos menor que 144 mg % o a 60 minutos menor que 160 mg %. Los niveles de glucosa sanguínea son determinados con un Glucometro Elite.

Con base en este análisis fenotípico, los animales se pueden distribuir a diferentes grupos experimentales. En particular, los animales con niveles de glucosa sanguínea más elevados se pueden asignar al grupo de tolerancia de glucosa deteriorada. Los ratones pueden ser alimentados sin restricción y se les puede proporcionar agua acidificada (pH 2.3).

Los ratones tolerantes y no tolerantes a glucosa pueden ser adicionalmente subdivididos en grupos de control, de proteínas de fusión del presente invento, y grupos de combinación de proteínas de fusión de albúmina/compuestos terapéuticos. Los ratones en el grupo de control pueden recibir una inyección intraperitoneal de vehículo diariamente, seis veces por semana. Los ratones en el grupo de fusión de albúmina pueden recibir una inyección intraperitoneal del terapéutico del presente invento (ej., proteínas de fusión de albúmina del invento y fragmentos y variantes de las mismas) en vehículo diariamente, seis veces por semana. Los ratones en el grupo de combinación de proteínas de fusión de albúmina/compuestos terapéuticos pueden recibir proteínas de fusión de albúmina y combinaciones de compuestos terapéuticos como se describió anteriormente.

El nivel de glucosa en orina en los ratones NOD se puede determinar sobre una base quincenal utilizando Labstix (Bayer Diagnostics, Hampshiere, England). El peso y absorción de fluidos también se puede determinar sobre una base quincenal. El comienzo de diabetes se define después de la aparición de glucosuria sobre dos determinaciones consecutivas. Después de 10 semanas de tratamiento, se puede realizar una IPGTT adicional y los animales se pueden sacrificar al día siguiente.

Sobre el curso de 10 semanas de tratamiento, los animales de control en ambos grupos tolerantes a glucosa y no tolerantes a glucosa desarrollan diabetes a una tasa de 60% y 86%, respectivamente (ver Patente Americana No. 5.866.546, Gross). Por lo tanto, altas tasas de

diabetes pueden ocurrir aún en ratones NOD inicialmente tolerantes a glucosa, si no se realiza intervención.

Los resultados se pueden confirmar mediante la medición de niveles de glucosa sanguínea en ratones NOD, antes y después de tratamiento. Los niveles de glucosa sanguínea son medidos como se describe anteriormente tanto en ratones tolerantes y no tolerantes a glucosa en todos los grupos descritos.

En una realización alternativa, los terapéuticos del presente invento (ej., fusiones específicas divulgadas como SEQ ID NO:Y y fragmentos y variantes de las mismas) se pueden cuantificar utilizando análisis espectrométrico y cantidades apropiadas de proteínas se pueden resuspender antes de inyección en 50 mu.l solución salina amortiguada por fosfato (PBS) por dosis. Dos inyecciones, con un intervalo de una semana entre las mismas, se pueden administrar subcutaneamente bajo la piel dorsal de cada ratón. El monitoreo se puede realizar en dos ocasiones separadas antes de inmunización y se puede realizar semanalmente durante el tratamiento y continuar después de este. La orina puede ser probada para glucosa cada semana (Keto-Diastix. RTM; Miles Inc., Kankakee, III.) y los ratones glicosúricos pueden ser revisados para glucosa de suero (ExacTech. RTM, MediSense, Inc., Waltham, Mass). La diabetes es diagnosticada cuando la glicemia en ayunas es mayor que 2.5 g/L.

EJEMPLO. 57: Examen Histológico de Ratones.

El examen histológico de muestras de tejidos de ratones NOD puede demostrar la capacidad de las composiciones del presente invento, y/o una combinación de las composiciones del presente invento con otros agentes terapéuticos para diabetes, para aumentar la concentración relativa de células beta en el páncreas. El método experimental es como sigue:

los ratones del ejemplo 56 se pueden sacrificar al final de período de tratamiento y las muestras de tejidos se pueden tomar del páncreas. Las muestras se pueden fijar en formalina al 10% y en solución salina al 0.9% e incrustar en cera. Dos grupos de 5 secciones seriales 5 mu.m se pueden cortar para inmunorotulación a un intervalo de corte de 150 .mu.m. Las secciones se pueden inmunorotular para insulina (dilución 1:1000 de antisueros anti-insulina de conejillo de indias, ICN Thames U.K.) y glucagon (dilución 1:2000 de antisueros de glucagon anti-pancreaticos de conejo) y detectar con antisueros anti-conejillo de indias peroxidasa conjugado (Dako, High Wycombe, U.K.) o anti-conejo peroxidasa conjugado (dilución 1:50, Dako).

La composición del presente invento puede o puede no tener un efecto tan fuerte sobre la masa visible de células beta como lo tiene sobre las manifestaciones clínicas de diabetes en animales tolerantes a glucosa y no tolerantes a glucosa.

EJEMPLO 58: Modelo de Ratón In Vivo de NIDDM

Ratones macho C57BL/6J de Jckson Laboratory (Bar Harbor, ME) se pueden obtener a las tres semanas de edad y alimentar con comida convencional o dietas enriquecidas en grasa (35.5% por peso; Bioserv. Frechtown NJ) o fructosa (60% por peso; Harlan Teklad, Madison, WI). La comida regular está compuesta de 4.5% por peso de grasa, 23% por peso de proteína, 31.9% por peso de almidón, 3.7% por peso de fructosa, y 5.3% por peso de fibra. La dieta alta en grasa (lardo) está compuesta de 35.5% por peso de grasa, 20% por peso de proteína, 36.4% por peso de almidón, 0.0% por peso de fructosa, y 0.1% por peso de fibra. La dieta alta en fructosa está compuesta de 5% por peso de grasa, 20% por peso de proteína, 0.0% por peso de almidón, 60% por peso de fructosa, y 9.4% por peso de fibra. Los ratones son albergados no más de cinco por jaula a 22ºC +/- 3ºC con humedad controlada de 50% +/- 20% con un ciclo de luz de 12 horas (6 am a 6 pm) (Luo, 1998, Metabolismo 47(6): 663-8, "Modelos de Ratón no genéticos de diabetes mellitus no dependiente de insulina"; Larsen, Diabetes 50(11): 2530-9 (2001), "La

administración sistémica del derivado GLP-1 de larga acción NN2211 induce perdida de peso durable y reversible tanto en ratas normales como obesas"). Después de exposición a dietas respectivas durante 3 semanas, los ratones pueden ser inyectados intraperitonealmente con estreptozotocina, "STZ" (Sigma, St. Louis, MO), a 100 mg/kg de peso corporal o vehículo (0.05 mol/L ácido cítrico, pH 4.5) y mantenidos en la misma dieta durante las próximas 4 semanas. Bajo condiciones en no ayunas, se obtuvo sangre 1, 2, y 4 semanas post-STZ al cortar la parte distal de la cola. Las muestras son utilizadas para medir las concentraciones de glucosa de plasma e insulina en no ayunas. El peso corporal y absorción de alimento son registrados semanalmente.

Para determinar directamente el efecto de la dieta alta en grasa sobre la capacidad de insulina para estimular eliminación de glucosa, se pueden iniciar experimentos sobre tres grupos de ratones, alimentados con grasa, alimentados con comida convencional inyectada con vehículo, y alimentados con grasa inyectada con STZ al final del periodo de 7 semanas descrito anteriormente. Los ratones pueden ser sometidos a ayuno durante cuatro horas antes de los experimentos. En la primera serie de experimentos, los ratones pueden ser anestesiados con inhalación de metoxiflurano (Pitman-Moor, Mndelein, IL). Insulina regular (Sigma) se puede inyectar intravenosamente ([IV] 0.1 U/kg de peso corporal) a través de la vena de la cola, y se puede recolectar sangre 3, 6, 9, 12, y 15 minutos después de la inyección de una vena de la cola diferente. Las concentraciones de glucosa de plasma se pueden determinar sobre estas muestras, y la vida media (t1/2) de desaparición de glucosa desde el plasma se puede calcular utilizando WinNonlin (Scientific Consulting, Apex, NC), un software de farmacocinética/farmacodinámica.

En la segunda serie de experimentos, los ratones pueden ser anestesiados con pentobarbital de sodio intraperitoneal (Sigma). La cavidad abdominal es abierta, y la vena abdominal principal es expuesta y cateterizada con un cateter IV calibre 24 (Johnson-Johnson Medical, Arlington, TX). El cateter es asegurado a tejido muscular adyacente a la vena abdominal, cortado en la parte inferior de la conexión de jeringa, y acoplado a un tubo plástico prellenado PE50, el cual a su vez es conectado a una jeringa con solución de infusión. Luego la cavidad abdominal es cerrada por sutura. Con este enfoque, no habría bloqueo de retroflujo de la sangre desde la parte inferior del cuerpo. Los ratones se pueden someter a infusión continua con glucosa (24.1 mg/kg/min) e insulina (10 mU/kg/min) a un volumen de infusión de 10 ul/min. Se pueden tomar muestras de sangre retro-orbital (70 uL cada una) 90, 105, 120, y 135 minutos después del comienzo de infusión para medición de concentraciones de glucosa de plasma e insulina. La media de estas cuatro muestras se utiliza para estimular concentraciones de glucosa de plasma de estado estable (SSPG) e insulina (SSPI) para cada animal.

Finalmente, los experimentos para evaluar la capacidad de proteínas de fusión de albúmina, las composiciones terapéuticas de la presente aplicación, ya sea solas o en combinación con cualquiera o más fármacos terapéuticos listados para el tratamiento de diabetes mellitus, para disminuir glucosa de plasma se pueden realizar en los dos grupos siguientes de modelos de ratones "NIDDM" que son STZ-inyectados: (1) alimenados con grasa C57BL/6J, y (2) alimentados con fructosa C57BL/6J. Las concentraciones de glucosa de plasma de los ratones para éstos estudios pueden estar en el rango entre 255 y 555 mg/dL. Los ratones son aleatoriamente asignados a tratamiento ya sea con vehículo, terapéutico de proteína de fusión del presente invento solo o en combinación con uno o más fármacos terapéuticos listados para el tratamiento de diabetes mellitus. Se pueden administrar un total de tres dosis. Se pueden tomar muestras de sangre de la vena de la cola para mediciones de concentración de glucosa de plasma antes de la primera dosis y tres horas después de la dosis final.

Las concentraciones de glucosa de plasma se pueden determinar utilizando el kit de diagnóstico de glucosa de Sigma (Sigma No. 315), un ensayo colorimétrico de enzimas. Los

niveles de insulina de plasma se pueden determinar utilizando el kit RIA de Insulina de Rata de Linco Research (#R1-13K; St. Charles, MO).

EJEMPLO 59: Ensayos de Reporteros H4IIe - SEAP in vitro Estableciendo Relación en Acción de Insulina.

Los Diferentes Reporteros H4II3

H41Ie/rMEP-SEAP: El promotor enzimático málico aislado a partir de rata (rMEP) contiene un elemento PPAR-gama el cual está en la ruta de infusión. Este constructo reportero es transfectado de manera estable hacia la línea celular hepática H4IIe.

H4Ie/SREBP-SEAP: La proteína de unión de elemento regulador de esterol (SREBP-1c) es un factor de transcripción que actúa sobre los promotores de un número de genes sensibles a insulina, por ejemplo, sintasa de ácido graso (FAS), y la cual regula la expresión de genes claves en metabolismo de ácidos grasos en fibroblastos, adipocitos, y hepatocitos. SREBP-1c, también conocido como el factor 1 de determinación y diferenciación de adipocitos (ADD-1), es considerado como el mediador principal de efectos de insulina sobre expresión de genes en células adiposas. Su actividad es modulada por los niveles de insulina, esteroles, y glucosa. Este constructo reportero es transfectado de manera estable hacia la línea celular hepática H4IIe.

H4Ie/FAS-SEAP: Los constructos reporteros de sintasa de ácidos grasos contienen un promotor FAS sensible a SREBP mínimo. Este constructo reportero es transfectado de manera estable hacia la línea celular hepática H4IIe.

H4Ie/PEPCK-SEAP: El promotor de carboxiquinasa de fosfoenolpiruvato (PEPCK) es el sitio principal de regulación hormonal de actividad PEPCK moduladora de transcripción de genes PEPCK. PEPCK cataliza un paso comprometido y limitante de tasa en gluconeogenesis hepática y por lo tanto se debe controlar cuidadosamente para mantener niveles de glucosa sanguínea dentro de límites normales. Este constructo reportero es transfectado de manera estable hacia la línea celular hepática H4IIe.

Estos constructos reporteros, también se pueden transfectar de manera estable hacia fibroblastos 3T3-L1 y mioblastos L6. Luego estas líneas celulares estables son diferenciadas en adipocitos 3T3-L1 y miotubos L6 como se describió anteriormente en el Ejemplo 13. Luego las líneas celulares diferenciadas se pueden utilizar en el ensayo SEAP descrito a continuación.

Medio de Crecimiento y Ensayo

El medio de crecimiento comprende Suero Bovino Fetal al 10% (FBS), Suero de Ternero al 10%, NEAA al 1%, 1x penecilina/estreptomicina, y 0.75 mg/mL G418 (para H4IIe/rFAS-SEAP y H4IIe/SREBP-SEAP) o 0.50 mg/mL G418 (para H4IIe/rMEP-SEAP). Para H$IIe/PEPCK-SEAP, el medio de crecimiento consta FBS al 10%, 1% penicilina/estreptomicina, 15 mM HEPES solución salina, 0.50 mg/mL G418.

El medio de ensayo consta de medio DMEM bajo en glucosa (Life Technologies), 1% NEAA, 1x penicilina/estreptomicina para los reporteros H4IIe/rFAS-SEAP, H4IIe/SREBP-SEAP, H411e/rMEP-SEAP. El medio de ensayo para el reportero H4IIe/PEPCK-SEAP consta de FBS al 0.1%, 1% penicilina/estreptomicina, y 15 mM HEPES solución salina amortiguado.

Metodo

Placas de 96 pozos son sembradas a 75.000 células/pozo en 100 uL de medio de crecimiento hasta que las células en la fase de crecimiento de registro se vuelven adherentes. Las células son cosechadas durante 48 horas al reemplazar medio de crecimiento con medio de ensayo 200 uL/pozo. (Para células H4IIe/PECK-SEAP, el medio de ensayo conteniendo 0.5 uM de dexametasona es agregado a 100 uL/pozo e incubado durante aproximadamente 20 horas). El medio de ensayo es reemplazado luego con 100 uL/pozo de medio de ensayo fresco, y una

alicuota de 50 uL de sobrenadante celular obtenido a partir de líneas celulares transfectadas expresando el terapéutico del presente invento (ej., proteínas de fusión de albúmina del invento y fragmentos y variantes de las mismas) es agregada al pozo. Los sobrenadantes de líneas celulares transfectadas de vectores vacíos son utilizados como control negativo. La adición de 10 nM y/o 100 nM insulina a los pozos se utiliza como control positivo. Después de 48 horas de incubación, los medios acondicionados son cosechados y la actividad SEAP medida (protocolo Phospha-Light System, Tropix #BP2500). Brevemente, las muestras son diluidas 1:4 en amortiguador de dilución e incubadas a 65°C durante 30 minutos para inactivar la forma no placental endógenas de SEAP. Una alicuota de 50 uL de las muestras diluidas es mezclada con 50 uL de Amortiguador de Ensayo SEAP el cual contiene una mezcla de inhibidores activos contra las isoenzimas SEAP no placentales y es incubada durante otros 5 minutos. Una alícuota de 50 ul de sustrato quimioluminiscente CSPD la cual es diluida 1:20 en mejorador de luminiscencia Emerald es agregada a la mezcla e incubada durante 15-20 minutos. Las placas son leídas en un luminometro de placas Dynex.

Ejemplo 60: Animales Transgénicos.

Las proteínas de fusión de albúmina del invento también se pueden expresar en animales transgénicos. Animales de cualquier especie, incluyendo, pero sin limitar a ratones, ratas, conejos, hamsters, conejillos de Indias, cerdos, micro-cerdos, cabras, ovejas, vacas y primates no humanos, ejemplo, mandriles, monos, y chimpancés se pueden utilizar para generar animales transgénicos. En una realización específica, las técnicas descritas en la presente de otra manera conocidas en el estado de la técnica, son utilizadas para expresar proteínas de fusión del invento en humanos, como parte de un protocolo de terapia genética.

Cualquier técnica conocida en el estado de la técnica puede ser utilizada para introducir polinucleotidos que codifican las proteínas de fusión de albúmina del invento en animales para producir las líneas fundadoras de animales transgénicos. Tales técnicas incluyen, pero no están limitadas a microinyección pronuclear (Paterson Appl. Microbiol. 40:691-698 (1994); Carver, Biotechnology (NY) 11:1263-1270 (1993); Wright, Biotecnology (NY) 9:830-834 (1991); y Hoppe, Patente Americana No. 4.873.191 (1989)); transferencia genética mediada por retrovirus hacia líneas germinales (Van der Putten, Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocistos o embriones; direccionamiento de genes en células madre embrionicas (Thompson, Cell 56:313-321 (1989)); electroporación de células o embriones (Lo, 1983, Mol Cell Biol. 3:1803-1814 (1983)); introducción de los polinucleotidos del invento utilizando una pistola de genes (ver, ej., Ulmer, Science 259:1745 (1993); introducción de constructos de ácidos nucleicos en células madre pleuripotente embrionico y transferencia de las células madre de vuelta hacia el blastocisto; y transferencia de genes mediada por esperma (Lavitrano, Cell 57:717-723 (1989); etc. Para una reseña de tales técnicas, ver Gordon, "Animales Transgénicos", Intl. Rev. Cytol 115:171-229 (1989), la cual es incorporada en la presente como referencia en su totalidad.

Cualquier técnica conocida en el estado de la técnica se puede utilizar para producir clones transgénicos que contienen polinucleotidos que codifican proteínas de fusión de albúmina de invento, por ejemplo, transferencia nuclear hacia oocitos enucleados de núcleos a partir de células embriónicas cultivadas, fetales, o adultas inducidas a estado de reposo (Campell, Nature 380:64-66 (1996); Wilmut, Nature 385:810-813 (1997)).

El presente invento proporciona animales transgénicos que llevan los polinucleotidos que codifican las proteínas de fusión de albúmina del invento en todas sus células, así como también animales que llevan estos polinucleotidos en algunas, pero no en todas sus células, es decir animales mosaicos o quiméricos. El transgen se puede integrar como un transgen simple o como múltiples copias tal como en concatémeros, ej., tandems cabeza a cabeza o tandems cabeza a

cola. El transgen se puede introducir selectivamente y activar en un tipo de célula particular al seguir, por ejemplo, la enseñanza de Lasko (lasko, Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). Las secuencias reguladoras requeridas para tal activación específica a tipo de célula dependerán del tipo de célula particular de interés, y será manifiestas a aquellos con experiencia en el estado de la técnica. Cuando se desea que el polinucleotido que codifica la proteión de fusión del invento sea integrado en el sitio cromosomal del gen endógeno correspondiente a la porción de proteína Terapéutica o porción de albúmina de la proteína de fusión del invento, se prefiere direccionamiento de genes. Brevemente, cuando tal técnica se va a utilizar, los vectores que contienen algunas secuencias nucleotidas homólogas al gen endógeno son diseñados para propósito de integrar, a través de recombinación homóloga con secuencias cromosomales, en y alterar la función de las secuencias nucleotidas del gen endógeno. El transgen también se puede introducir selectivamente en un tipo de célula particular, inactivando así es gen endógeno en solamente ese tipo de célula, al seguir, por ejemplo, la enseñanza de Gu (Gu, Science 265:103-106 (1994)). Las secuencias reguladoras requeridas para tal inactivación específica a tipo celular dependerán del tipo de célula particular de interés, y serán manifiestas a aquellos con experiencia en el estado de la técnica.

Una vez generados los animales transgénicos, la expresión del gen recombinante puede ser probada utilizando técnicas estándares. La selección inicial se puede realizar mediante análisis Southern blot o técnicas PCR para analizar tejidos de animales para verificar que la integración del polinucleotido que codifica la proteína de fusión del invento ha tenido lugar. El nivel de expresión mARN del polinucleotido que codifica la proteína de fusión del invento en los tejidos de animales transgénicos también se puede evaluar utilizando técnicas las cuales incluyen, pero no están limitadas a análisis Northern blot de muestras de tejidos obtenidas a partir del animal, análisis de hibridización *in situ*, y transcriptasa-PCR (rt-PCR) inversa. Muestras de tejido de expresión de proteínas de fusión también se pueden evaluar de manera inmunocitoquímica o inmunohistoquímica utilizando anticuerpos específicos para la proteína de fusión.

Una vez producidos los animales fundadores, estos pueden ser inbred, outbred, o crossbred para producir colonias del animal particular. Ejemplos de tales estrategias de cruce incluyen, pero no están limitados a: exogamia de animales fundadores con más de un sitio de integración para establecer líneas separadas; endogamia de líneas separadas para producir transgénicos compuestos que expresan el transgen a niveles mayores debido a los efectos de expresión aditiva de cada transgen; cruce de animales transgénicos heterocigosos para producir animales homocigosos para un sitio de integración dado para aumentar expresión y eliminar la necesidad de seleccionar animales mediante análisis e ADN; cruce de líneas homocigosas separadas para producir líneas compuestas heterocigosas u homocigosas; y cruce para colocar el transgen (es decir, polinucleotido que codifica una proteína de fusión de albúmina del invento) sobre un fondo distinto que es apropiado para un modelo experimental de interés.

Los animales transgénicos del invento tienen usos que incluyen, pero no están limitados a sistemas de modelos animales útiles en la elaboración de la función biológica de proteínas de fusión del invento y la proteína terpapéutica y/o componente de albúmina de la proteína de fusión del invento, estudiando condiciones y/o trastornos asociados con expresión aberrante, y en selección de compuestos efectivos para aliviar tales condiciones y/o trastornos.

EJEMPLO 61: Método de Tratamiento Utilizando Terapia Genética Ex Vivo.

Un método de terapía genética transplanta fibroblastos, los cuales son capaces de expresar una proteína de fusión de albúmina del presente invento, sobre un paciente. Generalmente, los fibroblastos son obtenidos de un sujeto mediante biopsia de piel. El tejido resultante es colocado en medio de cultivo de tejido y separado en piezas pequeñas. Trozos pequeños de tejido son

colocados sobre una superficie humeda de una matraz de cultivo de tejido, aproximadamente se colocan 10 piezas en cada matraz. La matraz es volteada al reves, cerrada de manera ajustada y dejada a temperatura ambiente durante la noche. Después de 24 horas a temperatura ambiente, la matraz es invertida y los trozos de tejido permanecen fijos al fondo de la matraz y se agrega medio fresco (ej., medio F12 de Ham, con FBS al 10%, penicilina y estreptomicina). Luego las matraces son incubadas a 37 grados C durante aproximadamente una semana.

En este momento, se agrega medio fresco y se cambio luego cada varios días. Después de dos semanas adicionales en cultivo, emerge una monocapa de fibroblastos. La monocapa es triptinizada y graduada a redomas más grandes.

pMV-7 (Kirschmeier, PT. ADN, 7:219-25 (1988)), flanqueada por repeticiones terminales largas del virus de sarcoma murino de Moloneu, es digerida con EcoR1 y HindIII y luego tratada con fosfatasa intestinal de ternero. El vector lineal es fraccionado sobre gel de agarosa y purificado, utilizando perlas de cristal.

Los polinucleotidos que codifican una proteína de fusión de albúmina del invento se pueden generar utilizando técnicas conocidas en el estado de la técnica amplificados utilizando cebadores PCR que corresponden a las secuencias finales 5' y 3' y ocasionalmente con sitios de restricción apropiados y codones de iniciación/detención, si es necesario. Preferiblemente, el cebador 5' contiene el sitio EcoRI y el cebador 5' incluye el sitio HindIII. Cantidades iguales del esqueleto lineal de virus de sarcoma murino de Moloney y el fragmento EcoRI y HindIII amplificados son agregados juntos, en presencia de ligasa de ADN T4. La mezcla resultante es mantenida bajo condiciones apropiadas para ligación de dos fragmentos. Luego la mezcla de ligación es utilizada para transformar bacterias HB101, las cuales son sembradas en placas sobre agar conteniendo canamicina para el propósito de confirmar que el vector tiene el gen de interés adecuadamente insertado.

Las células de empaque pA317 anfotrópicas o GP+am12 son desarrolladas en cultivo de tejidos a densidad confluente en Medio Eagles modificado de Dulbecco (DMEM) con suero de ternero al 10% (CS), penicilina y estreptomidina. Luego se agrega el vector MSV conteniendo el gen a los medios y las células de empaque son transducidas con el vector. Las células de empaque ahora producen particulas virales infecciosas conteniendo el gen (las células de empaque ahora son citadas como células productoras).

Se agrega medio fresco a las células productoras transducidas, y luego, el medio es cosechado desde una placa de 10 cm de células productoras confluentes. El medio gastado, conteniendo las particulas virales infecciosas, es filtrado a través de un filtro de miliporo para retirar las células productoras separadas y este medio luego se utiliza para infectar las células de fibroblastos. El medio es retirado a partir de una placa sub-confluente de fibroblastos y rapidamente es reemplazado con el medio a partir de las células productoras. Este medio es retirado y reemplazadocon medio fresco. Si el titulo del virus es alto, entonces virtualmente todos los fibroblastos serán infectados y no se requiere selección. Si el título es muy bajo, entonces es necesario utilizar un vector retroviral que tenga un marcador seleccionable, tal como neo o his. Una vez los fibroblastos han sido eficientemente infectados, los fibroblastos son analizados para determinar si la proteína de fusión de albúmina es producida.

Luego los fibroblastos diseñados son transplantados sobre el huésped, solos o después de haber sido cultivados hásta confluencia sobre perlas microtransportadoras cytodex 3.

EJEMPLO 62: Método de Tratamiento utilizando Terapia Genética - In Vitro.

Otro aspecto del presente invento es la utilización de métodos de terapia genética in vivo para tratar trastornos, enfermedades y condiciones. El método de terapía genética se relaciona con la introducción de secuencias de ácidos nucleicos desnudos (ADN, ARN, y ADN o ARN

antisentido) que codifican una proteína de fusión de albúmina del invento en un animal. Los polinucleotidos que codifican proteínas de fusión de albúmina del presente invento pueden estar operativamente ligados (es decir, asociados con) un promotor o cualquier otro elemento genético necesario para la expresión del polipéptido mediante el tejido objetivo. Tal terapia genética y técnicas de liberación y métodos son conocidos en el estado de la técnica, ver, por ejemplo, WO90/11092, WO98/11779; Patente Americana No. 5693622, 5705151, 5580859; Tabala, Cardiovasc. Res. 35(3): 470-479 (1997); Chao, Pharmacol. Res. 35(6):517-522 (1997); Wolff, Neuromusul. Disord. 7(5):314-318 (1997); Schwartz, Gene Ther. 3(5):405-411 (1996); Tsurumi, Circulation 94(12):3281-3290 (1996) (incorporadas en la presente como referencia).

Los constructos polinucleotidos se pueden liberar mediante cualquier método que libere materiales inyectables a las células de un animal, tal como, inyección en el espacio intersticial de tejidos (corazón, músculo, piel, pulmón, hígado, intestino y similares). Los constructos polinucleotidos se puede liberar en un vehículo acuoso o líquido farmacéuticamente aceptable.

El termino polinucleotido, ADN o ARN desnudo se refiere a secuencias que están libres de cualquier vehículo de liberación que actua para asistir, promover, o facilitar entrada en la célula, incluyendo secuencias virales, particulas virales, fórmulaciones de liposomas, lipofectina o agentes de precipitación y similares. Sin embargo, los polinucleotidos que codifican proteinas de fusión de albúmina del presente invento también se pueden liberar en fórmulaciones de liposomas (tales como aquellas enseñadas por Felgner P.L. (1995) Ann. NY Acad. Sci. 772:126-139 y Abdallah B. (1995) Biol. Cell 85(1):1-7) las cuales se pueden preparar mediante métodos bien conocidos por aquellos con experiencia en el estado de la técnica.

Los constructos de vectores polinucleotidos utilizados en el método de terapia genética preferiblemente son constructos que no se integraran en el genoma huésped ni contendrán secuencias que permiten replicación. Cualquier promotor fuerte conocido por aquellos con experiencia en el estado de la técnica puede ser utilizado para promover la expresión de ADN. Diferente de otras técnicas de terapia genética, una ventaja principal de introducir secuencias de ácido nucleico desnudo en células objetivo es la naturaleza transitoria de la síntesis polinucleotida en las células. Los estudios han mostrado que las secuencias de ADN no replicativas se pueden introducir en las células para proporcionar producción del polipeptido deseado durante periodos de hasta seis meses.

El constructo polinucleotido se puede liberar en el espacio intersticial de tejidos dentro de un animal, incluyendo músculo, piel, cerebro, pulmón, hígado, bazo, médula ósea, timo, corazón, linfa, sangre, hueso, cartílago, páncreas, riñón, vesícula biliar, estómago, intestino, testículos, ovario, útero, recto, sistema nervioso, ojos, glándulas, y tejido conectivo. El espacio intersticial de los tejidos comprende el fluido intercelular, matriz mucopolisacarida entre las fibras reticulares de tejidos de órganos, fibras elásticas en las paredes de los vasos o cámaras, fibras de colageno de tejidos fibrosos, o aquella misma matriz dentro de tejido conectivo envolviendo células musculares o en las lagunas de hueso. Este es de manera similar el espacio ocupado por el plasma de la circulación y el fluido linfático de los canales linfáticos. La liberación hacia el espacio intersticial de tejido muscular es preferida por razones discutidas a continuación. Se pueden liberar de manera conveniente mediante inyección en los tejidos que comprenden estas células. Se pueden liberar y expresar preferiblemente en células persistentes, no divisibles las cuales son diferenciadas, aunque la liberación y expresión se puede lograr en células no diferenciadas y menos completamente diferenciadas, tal como, por ejemplo, células madre de sangre o fibroblastos de piel. Las células musculares *in vivo* son particularmente competentes por su capacidad para absorber y expresar polinucleotidos.

Para inyección de polinucleotido desnudo, una cantidad de dosificación efectiva de ADN o ARN estará en el rango entre 0.05 g/kg y 50 mg/kg de peso corporal aproximadamente. Preferiblemente la dosificación estará entre 0.005 mg/kg y 20 mg/kg aproximadamente y más preferiblemente entre 0.05 mg/kg y 5 mg/kg aproximadamente. Naturalmente, como apreciará alguien con experiencia en el estado la técnica, esta dosificación variará según el sitio de inyección de tejidos. La dosificación apropiada y efectiva se puede determinar fácilmente por aquellos con experiencia común en el estado de la técnica y puede depender de la condición tratada y la ruta de administración. La ruta preferida de administración es la ruta parenteral de inyección en el espacio intersticial de tejidos. Sin embargo, también se pueden utilizar otras rutas parenterales, tales como, inhalación de una fórmulación en aerosol particularmente para liberación en los pulmones o tejidos bronquiales, garganta o membranas mucosas de la nariz. Además, los constructos polinucleotidos desnudos pueden ser liberados hacia arterias durante angioplastia mediante el catéter utilizado en el procedimiento.

Los efectos de respuesta de dosificación de polinucleotido inyectado en músculo *in vivo* es determinado como sigue. ADN plantilla apropiado para producción de mARN que codifica el polipéptido del presente invento es preparado según una metodología de ADN recombinante estándar. El ADN plantilla el cual puede ser circular o lineal, es utilizado como ADN desnudo o en complejo con liposomas. Luego los músculos cuadripceps de ratones son inyectados con varias cantidades del ADN plantilla.

Ratones Balb/C hembras y macho son anestesiados mediante inyección intraperitoneal con 0.3 ml de 2.5% de Avertina. Se realiza una incisión de 1.5 cm sobre el muslo anterior, y el músculo cuadriceps es directamente visualizado. El ADN plantilla es inyectado en 0.1 ml de vehículo en un jeringa de 1 cc a través de una aguja calibre 27 durante un minuto, aproximadamente 0.5 cm desde el sitio de inserción distal del músculo en la rodilla y aproximadamente 0.2 cm de profundidad. Se coloca una sutura sobre el sitio de inyección para localización futura, y la piel es cerrada con sujetadores de acero inoxidable.

Después de un tiempo de incubación apropiado (ej., 7 días) se preparan extractos musculares cortando todo el cuádriceps. Cada quinto 15 um de sección transversal del cuádriceps individual es histologicamente teñido para expresión de proteínas. Un curso de tiempo para expresión de proteínas de fusión puede ser hecho en una forma similar excepto que cuádriceps de ratones diferentes son cosechados en momentos diferentes. La persistencia de ADN en músculo después de la inyección se puede determinar mediante análisis Southern blot después de preparar ADN celular total y sobrenadantes HIRT de ratones inyectados y de control. Los resultados de la experimentación anterior en ratones se pueden utilizar para extrapolar dosis adecuadas y otros parámetros de tratamiento en humanos y otros animales que utilizan ADN desnudo.

EJEMPLO 63: Ejemplos Biológicos de Proteínas de Fusión del Invento

Ensayos de Astrocitos y Neuronales.

Las proteínas de fusión de albúmina del invento se pueden probar para actividad para promover supervivencia, crecimiento externo de axonas, o diferenciación fenotípica de células neuronales corticales para inducir la proliferación de células inmunopositivas de proteínas ácidas fibrilares gliales, astrocitos. La selección de células corticales para el bioensayo se basa en la expresión prevalente de FGF-1 y FGF-2 en estructuras corticales y en el aumento previamente reportado de superviviencia neuronal cortical que resulta de tratamiento FGF-2. Un ensayo de incorporación de timidina, por ejemplo, se puede utilizar para elucidar la actividad de una proteína de fusión de albúmina del invento sobre estas células.

Además, los reportes anteriores que describen los efectos biológicos de FGF-2 (FGF básico) sobre neuronas corticales o hipocampales *in vitro* han demostrado aumentos tanto en

supervivencia neuronal como en el crecimiento externo de axonas (Walicke, "El factor de crecimiento de fibroblastos promueve la superviviencia de neuronas hipocampales disociadas y aumenta la extensión de axonas." *Proc. Natl. Acad. Sci. USA 83:*3012-3016 (1986) ensayo incorporado en la presente como referencia en su totalidad). Sin embargo, los reportes de experimentos hechos sobre células PC-12 indican que estas dos respuestas no son necesariamente sinónimos y pueden depender no solo del FGF probado sino también de cuales receptores son expresados sobre las células objetivo. Utilizando el paradigma de cultivo neuronal cortical primario, la capacidad de una proteína de fusión de albúmina del invento para inducir crecimiento externo de axonas se puede comparar con la respuesta lograda con FGF-2 utilizando, por ejemplo, un ensayo de incorporación de timidina.

Ensayos de Fibroblastos y Células Endoteliales.

Fibroblastos de pulmón humano son obtenidos en Clonetics (San Diego, CA) y mantenidos en medio de crecimiento de Clonetics. Células endoteliales microvasculares dérmicas son obtenidas a partir de aplicaciones celulares (San Diego, CA). Para ensayos de proliferación, los fibroblastos de pulmón humano y células endoteliales microvasculares dérmicas se pueden cultivar a 5.000 células/pozo en una placa de 96 pozos durante un día en medio de crecimiento. Luego las células son incubadas durante un día en medio basal BSA 0.1%. Después de reemplazar el medio con medio fresco BSA 0.1%, las células son incubadas la proteína de fusión de prueba de las proteínas del invento durante 3 días. Se agrega Alamar Blue (Alamar Biosciences, Sacramento, CA) a cada pozo hasta una concentración final de 10%. Las células son incubadas durante 4 horas. La viabilidad celular es medida al leer en un lector de fluorescencia CytoFluor. Para los ensayos PGE2, los fibroblastos de pulmones humanos son cultivados a 5000 células/pozo en una placa de 96 pozos durante un día. Después de un cambio de medio a 0.1% medio basal BSA, las células son incubadas con FGF-2 o proteína de fusión del invento con o sin IL-1alfa durante 24 horas. Los sobrenadantes son recolectados y probados para PGE2 mediante un kit EIA (Cayman, Ann Arbor, MI). Para los ensayos IL-6, los fibroblastos de pulmún humano son cultivados a 5000 células/pozo en una placa de 96 pozos durante un día. Después de un cambio de medio a 0.1% medio basal BSA, las células son incubadas con FGF-2 o con o sin una proteína de fusión de albúmina del invento y/o IL-1alfa durante 24 horas. Los sobrenadantes son recolectados y probados para IL-6 mediante un kit ELISA (Endogen, Cambridge, MA).

Fibroblastos de pulmún humano son cultivados con FGF-2 o una proteína de fusión de albúmina del invento durante 3 días en medio basal antes de adición de Alamar Blue para evaluar efectos sobre crecimiento de los fibroblastos. FGF-2 debe mostrar una estimulación a 10 - 2500 ng/ml lo cual se puede utilizar para comparar estimulación con la proteína de fusión del invento.

Proliferación celular basada en incorporación de [3H]-timidina

El siguiente ensayo de incorporación de [3H]-timidina se puede utilizar para medir el efecto de una proteína terapéutica, ejemplo, proteínas de factor de crecimiento, sobre la proliferación de células tales como células de fibroblastos, células epiteliales o células musculares inmaduras.

Los cultivos sub-confluentes son detenidos en la fase G1 mediante una incubación de 18 h en medio libre de suero. Luego las proteínas terapéuticas son agregadas durante 24 h y durante las últimas 4h los cultivos son rotulados con [3H]-timidina, a una concentración final de 0.33 uM (25 Ci/mmol, Amersham, Arlington Heights, IL). La [3H]-timidina incorporada es precipitada con ácido acetico al 10% helado durante 24 h. Luego las células son enjuagadas con ácido tricloroacético al 10% helado y luego con agua helada. Después de lisis en 0.5 M NaOH, los lisados y enjuagues PBS (500 ml) son combinados, y la cantidad de radioactividad es medida.

Modelos de Parkinson.

La pérdida de función motora en la enfermedad de Parkinson es atribuida a una deficiencia de dopamina estriatal que resulta de la degeneración de las neuronas de proyección dopaminérgica nigrostrialtales. Un modelo animal para Parkinson que ha sido extensamente caracterizado involucra la administración sistémica de 1,2,3,6-tetrahidropiridina de 1-metil-4-fenilo (MPTP). En el CNS, MPTP es absorbida por astrocitos y catabolizada por monoamina oxidasa B a 1-metil-4-fenil-piridina (MPP+) y liberada. Subsecuentemente, MPP+ es activamente acumulada en neuronas dopaminérgicas mediante el transportador de recaptación de alta afinidad para dopamina. Luego MPP+ es concentrada en mitocondrias mediante el gradiente electroquímico y selectivamente inhibe difosfato de adenina de nicotidamida: ubiquinona oxidoreductionasa (complejo I), interfiriendo así con el transporte y eventualmente generando radicales de oxígeno.

Se ha demostrado en paradigmas de cultivos de tejidos que FGF-2 (FGF básico) tiene actividad trófica hacia neuronas dopaminérgicas nigrales (Ferrari, Dev. Biol. 1989). Recientemente, el grupo del Dr. Unsicker ha demostrado que la administración de FGF-2 en implantes de espuma de gel en el estriato resulta en la protección casi completa de neuronas dopaminérgicas nigrales a partir de la toxicidad asociada con exposición MPTP (otto y Unsicker, J. Neuroscience, 1990).

Basados en los datos con FGF-2, una proteína de fusión de albúmina del invento se puede evaluar para determinar si tiene una acción similar a aquella de FGF-2 para aumentar supervivencia neuronal dopaminérgica in vitro y también se puede probar *in vivo* para protección de neuronas dopaminérgicas en el estriato a partir del daño asociado con tratamiento MPTP. El efecto potencial de una proteína de fusión de albúmina del invento es examinado primero *in vitro* en un paradigma de cultivo de células neuronales dopaminérgicas. Los cultivos son preparados al dividir la placa de fondo de cerebro medio a partir de embriones de rata Wistar de 14 días de gestación. El tejido es disociado con tripsina y sembrado a una densidad de 200.000 células/cm2 sobre portaobjetos de vidrio recubiertos con poliortinina-laminina. Las células son mantenidas en medio Eagle modificado de Dulbecco y medio F12 conteniendo suplementos hormonales (N1). Los cultivos son fijados con paraformaldehido después de ocho días in vitro y son procesados para hidroxilasa de tirosina, un marcador específico para neuronas dopaminérgicas, teñido inmunohistoquímico. Los cultivos celulares disociados son preparados a partir de ratas embriónicas. El medio de cultivo es cambiado cada tercer día y los factores también son agregados en ese momento.

Puesto que las neuronas dopaminérgicas son aisladas de animales el día 14 de gestación, un tiempo de desarrollo el cual es anterior a la etapa cuando las células precursoras dopaminérgicas están proliferando, un aumento en el número de neuronas inmunopositivas de tirosina hidroxilasa representaría un aumento en el número de neuronas dopaminérgicas sobreviviendo *in vitro*. Por lo tanto, si una proteína terapéutica del invento actúa para prolongar la supervivencia de neuronas dopaminérgicas, esto indicaría que la proteína de fusión estaría involucrada en la enfermedad de Parkinson.

EJEMPLO 64: Terapia de Combinación de Transplante de Células Beta pancreáticas.

El transplante es una forma común de tratamiento de enfermedad autoinmune, especialmente cuando el auto tejido objetivo ha sido dañado de manera severa. Por ejemplo, y no a manera de limitación, el transplante de páncreas y transplante de células de isletas son opciones de tratamiento comunes para IDDM (Ver, ej., Stewart, Journal of Clinical Endocrinology & Metabolism 86 (3): 984-988 (2001); Brunicardi, Transplant. Proc. 28: 2138-40 (1996); Kendall & Robertson, Diabetes Metab. 22: 157-163 (1996); Hamano, Kobe J. Med. Sci. 42: 93-104 (1996); Larsen & Stratta, Diabetes Matab. 22: 139-146 (1996); y Kinkhawala, Am. J. Surg. 171: 516-520

(1996)). Como con cualquier método de transplante, las terapias de transplante para pacientes con enfermedades autoinmunes incluyen tratamientos para minimizar el riesgo de rechazo de huesped del tejido trasplantado. Sin embargo, la enfermedad autoinmune involucra riesgo independiente adicional que la respuesta autoinmune de huesped preexistente la cual dañó el auto tejido originalejercerá el mismo efecto dañino sobre el tejido transplantado. Por consiguiente, el presente invento contempla métodos y composiciones para el tratamiento de enfermedades pancreáticas autoinmunes utilizando las proteínas de fusión de albúmina del presente invento en combinación con inmunomoduladores/inmunosupresores en individuos sometidos a terapia de transplante de la enfermedad autoinmune.

Según el invento, las composiciones y fórmulaciones basadas en fusión de albúmina descritas anteriormente, son administradas para prevenir y tratar daño a órganos, tejido, o células trasplantadas que resulta a partir de la respuesta autoinmune del individuo huesped inicialmente dirigida contra el auto-tejido original. La administración se puede realizar antes y después del transplante en 2 a 4 dosis cada una con intervalo de una semana entre éstas.

Los siguientes inmunomoduladores/inmunosupresores incluyendo, pero sin limitar a AI-401, CDP-571 (anticuerpo monoclonal anti-TNF), CG-1088, Diamyd (vacuna para la diabetes), ICM3 (anticuerpo monoclonal anti-ICAM-3), linomida (Roquinimex), NBI-6024 (ligando péptido alterado), TM-27, VX-740 (HMR-3480), inhibidores de caspasa 8 proteasa, talidomida, hOKT3gama1 (Ala-ala) (anticuerpo monoclonal anti-CD3), Interferón-Alfa-Oral, lactobacilus oral, y LymphoStat-B se pueden utilizar junto con los terapéuticos de fusión de albúmina del presente invento en transplante de células de isletas o pancreas.

EJEMPLO 65: Identificación y Clonación de Dominios VH y VL.

Un método para identificar y clonar dominios VH y VL a partir de líneas celulares expresando un anticuerpo particular es realizar PCR con cebadores VH y VL específicos sobre cADN hecho del anticuerpo que expresa las líneas celulares. Brevemente, ARN se aisla a partir de las líneas celulares y se utiliza como un modelo para RT-PCR diseñado para amplificar dominios VH y VL de los anticuerpos expresados por las líneas celulares EBV. Las células se pueden someter a lisis en el reactivo TRIzol (Life Technologies, Rockville. MD) y extraer con un quinto del volumen de cloroformo. Después de la adición de cloroformo, la solución es dejada incubar a temperatura ambiente durante 10 minutos, y luego centrifugada a 14.000 rpm durante 15 minutos a 4°C en una centrifuga. El sobrenadante es recolectado y el ARN es precipitado utilizando un volumen igual se isopropanol. El ARN precipitado es peletizado a 14.000 rpm durante 15 minutos a 4°C en una centrifuga. Después de centrifugación, el sobrenadante es descartado y lavado con etanol al 75%. Después de lavado, el ARN es centrifugado nuevamente a 800 rpm durante 5 minutos a 4°C. El sobrenadante es descartado y el pellet se deja secar al aire. El ARN es disuelto en agua DEPC y se calienta a 60°C durante 10 minutos. Las cantidades de ARN se pueden determinar utilizando mediciones de densidad óptica.

cADN se puede sintetizar, según métodos bien conocidos en el estado de la técnica, a partir de 1.5-2.5 microgramos de ARN utilizando transciptasa inversa y cebadores de hexamero aleatorios. Luego se utiliza cADN como un modelo para amplificación PCR de dominios VH y VL. Los cebadores utilizados para amplificar genes VH y VL son mostrados en la tabla 7. Típicamente una reacción PCR hace uso de un único cebador 5' y un único sebador 3'. Algunas veces, cuando la cantidad de genes son mostrados en la tabla 7. Típicamente una reacción PCR hace uso de un único cebador 5' y un único cebador 3'. Algunas veces, cuando la cantidad de ARN plantilla disponible es limitada, o para mayor eficiencia, se pueden utilizar grupos de cebadores 5' y/o 3'. Por ejemplo, algunas veces todos los cinco cebadores VH-5' y todos los cebadores JH3' son utilizados en una única reacción PCR. La reacción PCR es realizada en un

volumen de 50 microlitros conteniendo amortiguador PCR 1X, 2mM de cada dNTP, 0.7 unidades de polimerasa Taq de Alta Fidelidad, mezcla de cebadores 5', mezcla de cebadores 3' y 7.5 microlitros de cADN. La mezcla de cebadores 5' y 3' de VH y VL se puede realizar combinando 22 pmol y 28 pmol, respectivamente, de cada uno de los cebadores individuales. Las condiciones PCR son: 96°C durante 5 minutos; seguido por 25 ciclos de 94°C durante 1 minuto, 50°C durante 1 minuto, y 72°C durante 1 minuto; seguido por un ciclo de extensión de 72°C durante 10 minutos. Después de terminar la reacción, los tubos de muestra son almacenados a 4°C.

Tabla 7: Secuencias Cebadoras Utilizadas para Amplificar Dominios VH y VL.

Nombre del Cebador	SEQ ID NO	Secuencia cebadora (5`-3`)
VH		
Hu VH1-5`	62	CAGGTGCAGCTGGTGCAGTCTGG
Hu VH2-5'	63	CAGGTCAACTTAAGGGAGTCTGG
Hu VH3-5'	64	GAGGTGCAGCTGGTGGAGTCTGG
Hu VH4-5'	65	CAGGTGCAGCTGCAGGAGTCGGG
Hu VH5-5'	66	GAGGTGCAGCTGTTGCAGTCTGC
Hu. VH6-5`	67	CAGGTACAGCTGCAGCAGTCAGG
Hu VH7-5'	68	TGAGGAGACGGTGACCAGGGTGCC
Hu JH1,2-5'	69	TGAAGAGACGGTGACCATTGTCCC
Hu JH4,5-5'	70	TGAGGAGACGGTGACCAGGGTTCC
Hu JH6-5'	71	TGAGGAGACGGTGACCGTGGTCCC
Hu Vkappa11-5'	72	GACATCCAGATGACVVAGTCTCC
Hu Vkappa2a-5'	73	GATGTTGTGATGACTCAGTCTCC
Hu Vkappa2b-5`	74	GATATTGTGATGACTCAGTCTCC
Hu Vkappa3-5'	75	GAAATTGTGTTGACGCAGTCTCC
Hu Vkappa4-5`	76	GACATCGTGATGACCCAGTCTCC
Hu Vkappa5-5'	77	GAAACGACACTCACGCAGTCTCC
Hu Vkappa6-5'	78	GAAATTGTGCTGACTCAGTCTCC
Hu Vlambda11-5'	79	CAGTCTGTGTTGACGCAGCCGCC
Hu Vlambda2-5'	80	CAGTCTGCCCTGACTCAGCCTGC
Hu Vlambda3-5'	81	TCCTATGTGCTGACTCAGCCACC
Hu Vlambda3b-5`	82	TCTTCTGAGCTGACTCAGGACCC
Hu Vlambda4-5`	83	CACGTTATACTGACTCAACCGCC
Hu Vlambda5-5'	84	CAGGCTGTGCTCACTCAGCCGTC
Hu Vlambda6-5`	85	AATTTTATGCTGACTCAGCCCCA
Hu Jkappa1-3'	86	ACGTTTGATTTCCACCTTGGTCCC
Hu Jkappa2-3`	87	ACGTTTGATCTCCAGCTTGGTCCC
Hu Jkappa3-3`	88	ACGTTTGATATCCACTTTGGTCCC
Hu Jkappa4-3`	89	ACGTTTGATCTCCACCTTGGTCCC
Hu Jkappa15-3`	90	ACGTTTAATCTCCAGTCGTGTCCC
Hu Jlambda1-3`	91	CAGTCTGTGTTGACGCAGCCGCC
Hu Jlambda2-3`	92	CAGTCTGCCCTGACTCAGCCTGC
Hu Jlambda3-3`	93	TCCTATGTGCTGACTCAGCCACC
Hu Jlambda3b-3`	94	TCTTCTGAGCTGACTCAGGACCC
Hu Jlambda4-3`	95	CACGTTATACTGACTCAACCGCC
Hu Jlambda5-3`	96	CAGGCTGTGCTCACTCAGCCGTC
Hu Jlambda6-3`	97	AATTTTATGCTGACTCAGCCCCA

Luego las muestras PCR son sometidas a electroforesis sobre un gel de □ubcutá al 1.3%. Bandas de ADN de los tamaños esperados (506 pares base para dominios VH, y 344 pares base para dominios VL) se pueden cortar del gel y purificar utilizando métodos bien conocidos en el estado de la técnica. Los productos PCR purificados se pueden ligar a un vector de clonación PCR (vector TA de Invitrogen Inc., Carlsbad, Ca). Productos PCR clonados individuales pueden ser aislados después de transfección de E. coli y selección de color azul/blanco. Luego los productos PCR clonados pueden ser secuenciados utilizando métodos comúnmente conocidos en el estado de la técnica.

Las bandas PCR conteniendo el dominio VH y los dominios VL también se pueden utilizar para crear vectores de expresión Ig de longitud completa. Los dominios VH y VL se pueden clonar en vectores que contienen las secuencias □ubcutáneam de regiones constantes de cadena pesada (ej., IgG1 humano o IgG4 humano) o cadena liviana (kappa humana o lambda humana) tal que una molécula de cadena pesada o liviana completa pueda ser expresada a partir de estos vectores cuando es transfectada a una célula □ubcutá apropiada. Además, cuando cadenas pesadas y livianas clonadas son expresadas en una línea celular (a partir de uno o dos vectores), se pueden ensamblar en una molécula de anticuerpo funcional completa que es secretada hacia el medio de cultivo de células. Los métodos que utilizan □ubcutáneamente que codifican dominios de anticuerpos VH y VL para generar vectores de expresión que codifican moléculas de anticuerpos enteros son bien conocidos en el estado de la técnica.

EJEMPLO 66: Preparación de Proteínas de Fusión de Citoquinas-HA o Factor de Crecimiento-HA (tales como NGF, BDNFa, BDNFb y BDNFc).

El cADN para la citoquina o factor de crecimiento, tal como NGF, se puede aislar mediante una variedad de medios incluyendo a partir de bibliotecas cADN, mediante RT-PCR y mediante PCR utilizando una serie de cebadores □ubcutáneamente□ sintéticos de superposición, todos utilizando métodos estándares. Las secuencias □ubcutáneam para todas estas proteínas son conocidas y disponibles. El cADN se puede adaptar en los extremos 5' y 3' para generar sitios de restricción, tal que se pueden utilizar enlazadores □ubcutáneamente□, para clonar el cADN en un vector que contiene el cADN para HA. Esto puede ser en el término N o C con o sin el uso de una secuencia espaciadora. cADN de NGF (u otra citoquina) es clonado en un vector tal como pPPC005 (Figura 2), sScCHSA, pScNHSA, o pC4:HSA a partir del cual el casete de expresión completo luego es cortado e insertado en el plásmido pSAAC35 para permitir la expresión de la proteína de fusión de albúmina en levadura. La proteína de fusión de albúmina secretada a partir de levadura luego puede ser recolectada y purificada a partir del medio y probada para su actividad biológica. Para expresión en líneas celulares mamíferas, un procedimiento celular es adoptado excepto que el casete de expresión utilizado emplea un promotor mamífero, secuencia guía y terminador (Ver Ejemplo 1). Luego este casete de expresión es cortado e insertado en un plásmido apropiado para la transfección de líneas celulares mamíferas.

EJEMPLO 67: Preparación de proteínas de fusión HA-IFN (tal como IFN-alfa).

El cADN para la interferón de interés tal como IFNalfa se puede aislar mediante una variedad de medios incluyendo pero no exclusivamente, a partir de □ubcutáneam de cADN, mediante RT-PCR y mediante PCR utilizando una serie de cebadores □ubcutáneamente□ sintéticos de sobreposición, todos utilizando métodos estándares. Las secuencias oligonucleotidas para interferones, tales como INFalfa son conocidas y disponibles, por ejemplo, en Patentes US 5.326.859 y 4.588.585, en EP 32 134, así como también en bases de datos públicas tales como GenBank. El cADN puede ser adaptado en los extremos 5' y 3' para generar sitios de restricción,

tal que los enlazadores □ubcutáneamente se pueden utilizar para clonar el cADN en un venctor que contiene el cADN para HA. Esto puede ser en el término N o C de la secuencia HA, con o sin el uso de una secuencia espaciadora. El cADN de INFalfa (u otra interferón) es clonado en un vector tal como pPPC0005 (Figura 2), pScCHSA, pScNHSA, o pC4:HSA a partir del cual el casete de expresión completo luego es cortado e insertado en el plásmido pSAAC35 para permitir la expresión de la proteína de fusión de albúmina en levadura. La proteína de fusión de albúmina secretada a partir de levadura luego puede ser recolectada y purificada a partir del medio y probada para su actividad biológica. Para expresión en líneas celulares mamíferas, un procedimiento celular es adoptado excepto que el casete de expresión utilizado emplea un promotor mamífero, secuencia guía y terminador (Ver Ejemplo 1). Luego este casete de expresión es cortado e insertado en un plásmido apropiado para la transfección de líneas celulares mamíferas.

Recuperación máxima de proteínas a partir de recipientes

Las proteínas de fusión de albúmina de invento tiene un alto grado de estabilidad aún cuando están empacadas a bajas concentraciones. Además, a pesar de la baja concentración de proteínas, se observó una buena recuperación de proteínas de fusión aún cuando la solución acuosa no incluye otra proteína agregada para minimizar unión a las paredes del recipiente. La recuperación de soluciones HA-IFN almacenadas en recipientes se comparó con una solución concentrada. Soluciones HA-IFN de 6 o 30 ug/ml fueron colocadas en recipientes y almacenadas a 4°C. Después de 48 o 72 horas un volumen originalmente equivalente a 10 ng de muestra fue retirado y medido en un □ubcután IFN ELISA. Los valores estimados fueron comparados con aquel de una concentración alta de solución concentrada. Como se muestra, esencialmente no hay pérdida de la muestra en estos recipientes, indicando que la adición de material exógeno tal como albúmina no es necesaria para evitar pérdida de muestra hacia la pared de los recipientes.

Estabilidad in vivo y biodisponibilidad de fusiones HA-alfa-IFN

Para determinar la estabilidad in vivo y biodisponibilidad de una molécula de fusión HA-alfa-IFN, la molécula de fusión purificada (a partir de levadura) se administro a monos. Las composiciones farmacéuticas fórmuladas a partir de fusiones HA-alfa-IFN pueden explicar la biodisponibilidad y vida media de suero extendida y biodisponibilidad. Por consiguiente, las composiciones farmacéuticas se pueden fórmular para contener dosis menores de actividad de interferón alfa en comparación con la □ubcután interferón alfa nativa.

Las composiciones farmacéuticas que contienen fusiones HA-alfa-IFN se pueden utilizar para tratar o prevenir enfermedades en paciente con cualquier enfermedad o estado patológico que se puede modular mediante la administración de alfa-INF. Tales enfermedades incluyen, pero no están limitadas a leucemia de células pilosas, sarcoma de Kaposi, verrugas genitales y anales, hepatitis B crónica, hepatitis crónica no A, no B, en particular hepatitis C, hepatitis D, leucemia mielógena crónica, carcinoma de células renales, carcinoma de vejiga, carcinoma ovárico y cervical, cánceres de piel, papilomatosis respiratorio recurrente, linfoma no-Hodgkin y línfomas de células T cutaneas, melanoma, mieloma múltiple, SIDA, esclerosis múltiple, glioblastoma, etc (ver Interferón-alfa, En: HFS Drug Information, 1997.

Por consiguiente, el invento incluye composiciones farmacéuticas que contienen una proteína de fusión HA-alfa-IFN, polipéptido o péptido fórmulado con la dosificación adecuada para administración humana. El invento también incluye métodos para tratar pacientes en necesidad de tal tratamiento comprendiendo al menos el paso de administrar una composición sanguínea que contiene al menos una proteína de fusión Ha-alfa-IFN, polipéptido o péptido.

Fusiones HA-alfa-IFN bifuncionales

Un vector de expresión HA-alfa-IFN se puede modificar para incluir una inserción para la expresión de proteínas de fusión HA-alfa-IFN bifuncionales. Por ejemplo, el cADN para una secunda proteína de interés puede ser insertado en marco corriente sanguínea la secuencia "rHA-IFN" después que el codón de detención doble ha sido retirado o cambiado corriente sanguínea la secuencia codificadora.

En una versión de una proteína de fusión HA-alfa-IFN bifuncional, un anticuerpo o fragmento contra proteína estimuladora de linfocitos B (Acc 4455139) o polipéptido se puede fusionar a un extremo del componente HA de la □ubcután de fusión. Esta proteína bifuncional es útil para modular cualquier respuesta inmune generada por el componente alfa-IFN de la fusión.

EJEMPLO 68: □ubcutáne de Proteína de Fusión de hormona HA.

El cADN para la hormona de interés se puede aislar mediante una variedad de medios incluyendo pero no exclusivamente a partir de bibliotecas de cADN, mediante RT-PCR y mediante PCR utilizando una serie de cebadores oligosintéticos de sobreposición, todos utilizando métodos estándares. Las secuencias □ubcutáneam para todos estas proteínas son conocidas y disponibles, por ejemplo, en bases de datos públicas tales como GenBank. El cADN se puede adaptar en los extremos 5' y 3' para generar sitios de restricción, tal que se pueden utilizar enlazadores □ubcutáneamente□, para clonar el cADN en un vector que contiene el cADN para HA. Esto puede ser en el término N o C con o sin el uso de una secuencia espaciadora. La hormona cADN es clonada en un vector tal como pPPC0005 (Figura 2), sScCHSA, pScNHSA, o pC4:HSA a partir del cual el casete de expresión completo luego es cortado e insertado en el plásmido pSAAC35 para permitir la expresión de la proteína de fusión de albúmina en levadura. La proteína de fusión de albúmina secretada a partir de levadura luego puede ser recolectada y purificada a partir del medio y probada para su actividad biológica. Para expresión en líneas celulares mamíferas, un procedimiento celular es adoptado excepto que el casete de expresión utilizado emplea un promotor mamífero, secuencia guía y terminador (Ver Ejemplo 1). Luego este casete de expresión es cortado e insertado en un plásmido apropiado para la transfección de líneas celulares mamíferas.

EJEMPLO 69: Preparación de Receptor soluble en HA o proteína de fusión de proteína de unión de HA.

El cADN para el receptor soluble o proteína de unión de interés se puede aislar mediante una variedad de medios incluyendo pero no exclusivamente a partir de bibliotecas de cADN, mediante RT-PCR y mediante PCR utilizando una serie de cebadores oligosintéticos de sobreposición, todos utilizando métodos estándares. Las secuencias □ubcutáneam para todas estas proteínas son conocidas y disponibles, por ejemplo, en bases de datos públicas tales como GenBank. El cADN se puede adaptar en los extremos 5' y 3' para generar sitios de restricción, tal que se pueden utilizar enlazadores □ubcutáneamente□, para clonar el cADN en un vector que contiene el cADN para HA. Esto puede ser en el término N o C con o sin el uso de una secuencia espaciadora. El cADN receptor es clonado en un vector tal como pPPC0005 (Figura 2), sScCHSA, pScNHSA, o pC4:HSA a partir del cual el casete de expresión completo luego es cortado e insertado en el plásmido pSAAC35 para permitir la expresión de la proteína de fusión de albúmina en levadura. La proteína de fusión de albúmina secretada a partir de levadura luego puede ser recolectada y purificada a partir del medio y probada para su actividad biológica. Para expresión en líneas celulares mamíferas, un procedimiento celular es adoptado excepto que el casete de expresión utilizado emplea un promotor mamífero, secuencia guía y terminador (Ver Ejemplo 1). Luego este casete de expresión es cortado e insertado en un plásmido apropiado para la transfección de líneas celulares mamíferas.

EJEMPLO 70: Preparación de factores de crecimiento de HA.

El cADN para el factor de crecimiento se puede aislar mediante una variedad de medios incluyendo pero no exclusivamente a partir de bibliotecas de cADN, mediante RT-PCR y mediante PCR utilizando una serie de cebadores oligosintéticos de sobreposición, todos utilizando métodos estándares (ver GenBank Acc. No. NP_000609). El cADN se puede adaptar en los extremos 5' y 3' para generar sitios de restricción, tal que se pueden utilizar enlazadores □ubcutáneamente□, para clonar el cADN en un vector que contiene el cADN para HA. Esto puede ser en el término N o C con o sin el uso de una secuencia espaciadora. El cADN de factor de crecimiento es clonado en un vector tal como pPPC0005 (Figura 2), sScCHSA, pScNHSA, o pC4:HSA a partir del cual el casete de expresión completo luego es cortado e insertado en el plásmido pSAAC35 para permitir la expresión de la proteína de fusión de albúmina en levadura. La proteína de fusión de albúmina secretada a partir de levadura luego puede ser recolectada y purificada a partir del medio y probada para su actividad biológica. Para expresión en líneas celulares mamíferas, un procedimiento celular es adoptado excepto que el casete de expresión utilizado emplea un promotor mamífero, secuencia guía y terminador (Ver Ejemplo 1). Luego este casete de expresión es cortado e insertado en un plásmido apropiado para la transfección de líneas celulares mamíferas.

EJEMPLO 71: Preparación de proteínas de fusión de anticuerpos de cadena simple de HA.

Los anticuerpos de cadena simple se producen mediante diferentes métodos incluyendo pero sin limitar a: selección de bibliotecas de fagos, clonación de la región variable de un anticuerpo específico mediante clonación del cADN del anticuerpo y utilizando las regiones constantes de flanqueo como el cebador para clonar la región variable, o al sintetizar un oligonucleotido que corresponde a la región variable de cualquier anticuerpo específico. El cADN se puede adaptar en los extremos 5' y 3' para generar sitios de restricción, tal que se pueden utilizar enlazadores □ubcutáneamente□, para clonar el cADN en un vector que contiene el cADN para HA. Esto puede ser en el término N o C con o sin el uso de una secuencia espaciadora. El cADN de células es clonado en un vector tal como pPPC0005 (Figura 2), sScCHSA, pScNHSA, o pC4:HSA a partir del cual el casete de expresión completo luego es cortado e insertado en el plásmido pSAAC35 para permitir la expresión de la proteína de fusión de albúmina en levadura.

En moléculas de fusión del invento, VH y VL pueden ser enlazados por uno de los siguientes medios o una combinación de los mismos: un enlazador péptido entre el término C de VH y el término N de VL; un sitio de división de proteasa Kex2p entre el VH y VL tal que los dos son separados ante secreción y luego se auto asocian; y residuos de cistina posicionados tal que el VH y VL pueden formar un enlace de disulfuro entre ellos para unirlos. Una opción alternativa sería colocar el VH en el término N de HA o un fragmento de dominio HA y el VL en el término C del HA o fragmento de dominio HA.

La proteína de fusión de albúmina secretada a partir de levadura luego puede ser recolectada y purificada a partir del medio y probada para su actividad. Para expresión en líneas celulares mamíferas, un procedimiento celular es adoptado excepto que el casete de expresión utilizado emplea un promotor mamífero, secuencia guía y terminador (Ver Ejemplo 1). Luego este casete de expresión es cortado e insertado en un plásmido apropiado para la transfección de líneas celulares mamíferas. El anticuerpo producido en esta forma puede ser purificado a partir del medio y probado para su unión a su antígeno utilizando métodos inmunoquímicos estándares.

EJEMPLO 72: Preparación de proteínas de fusión de moléculas de adhesión de células de HA.

El cADN para la molécula de adhesión de células se puede aislar mediante una variedad de medios incluyendo pero no exclusivamente a partir de bibliotecas de cADN, mediante RT-PCR y mediante PCR utilizando una serie de cebadores oligosintéticos de sobreposición, todos utilizando métodos estándares. Las secuencias □ubcutáneam para todas estas proteínas son conocidas y disponibles, por ejemplo, en bases de datos públicas tales como GenBank. El cADN se puede adaptar en los extremos 5' y 3' para generar sitios de restricción, tal que se pueden utilizar enlazadores □ubcutáneamente□, para clonar el cADN en un vector que contiene el cADN para HA. Esto puede ser en el término N o C con o sin el uso de una secuencia espaciadora. El cADN de □ubcután de adhesión de células es clonado en un vector tal como pPPC0005 (Figura 2), sScCHSA, pScNHSA, o pC4:HSA a partir del cual el casete de expresión completo luego es cortado e insertado en el plásmido pSAAC35 para permitir la expresión de la proteína de fusión de albúmina en levadura. La proteína de fusión de albúmina secretada a partir de levadura luego puede ser recolectada y purificada a partir del medio y probada para su actividad biológica. Para expresión en líneas celulares mamíferas, un procedimiento celular es adoptado excepto que el casete de expresión utilizado emplea un promotor mamífero, secuencia guía y terminador (Ver Ejemplo 1). Luego este casete de expresión es cortado e insertado en un plásmido apropiado para la transfección de líneas celulares mamíferas.

EJEMPLO 73: Preparación de Factores Inhibitorios y péptidos como proteínas de fusión HA (tal como antivirales HA, anticuerpos HA, inhibidores de enzimas HA y proteínas anti-alérgicas HA).

El cADN del péptido de interés se puede aislar mediante una variedad de medios incluyendo pero no exclusivamente a partir de bibliotecas de cADN, mediante RT-PCR y mediante PCR utilizando una serie de cebadores oligosintéticos de sobreposición, todos utilizando métodos estándares. El cADN se puede adaptar en los extremos 5' y 3' para generar sitios de restricción, tal que se pueden utilizar enlazadores □ubcutáneamente□, para clonar el cADN en un vector que contiene el cADN para HA. Esto puede ser en el término N o C con o sin el uso de una secuencia espaciadora. El cADN de péptidos es clonado en un vector tal como pPPC0005 (Figura 2), sScCHSA, pScNHSA, o pC4:HSA a partir del cual el casete de expresión completo luego es cortado e insertado en el plásmido pSAAC35 para permitir la expresión de la proteína de fusión de albúmina en levadura. La proteína de fusión de albúmina secretada a partir de levadura luego puede ser recolectada y purificada a partir del medio y probada para su actividad biológica. Para expresión en líneas celulares mamíferas, un procedimiento celular es adoptado excepto que el casete de expresión utilizado emplea un promotor mamífero, secuencia guía y terminador (Ver Ejemplo 1). Luego este casete de expresión es cortado e insertado en un plásmido apropiado para la transfección de líneas celulares mamíferas.

EJEMPLO 74: Preparación de proteínas de fusión HA dirigidas.

El cADN para la proteína de interés se puede aislar a partir una biblioteca de cADN o se puede preparar □ubcutáneament utilizando varios □ubcutáneamente□ de sobreposición utilizando métodos de biología molecular estándares. Los sanguínea apropiados se pueden diseñar en el cADN para formar sitios de restricción convenientes y también para permitir la fijación del cADN de proteína al cADN de albúmina. También una proteína objetivo o cADN de péptido tal como anticuerpos o péptidos de cadena simple, tales como señales de localización nuclear, que pueden dirigir proteínas dentro de las células se pueden fusionar al otro extremo de albúmina. La proteína de interés y el péptido objetivo es clonado en un vector tal como pPPC0005 (Figura 2), sScCHSA, pScNHSA, o pC4:HSA el cual permite la fusión con cADN de albúmina. En esta forma tanto el extremo N- como C-terminal de albúmina son fusionados a otras proteínas. Luego el cADN fusionado es cortado a partir de pPPC0005 y es insertado en un

plásmido tal como pSAC35 para permitir la expresión de la proteína de fusión de albúmina en levadura. Todos los anteriores procedimientos pueden ser realizados utilizando métodos estándares en biología molecular. La proteína de fusión de albúmina secretada a partir de levadura puede ser recolectada y purificada a partir del medio y probada para su actividad biológica y su actividad de direccionamiento utilizando pruebas bioquímicas y biológicas apropiadas.

EJEMPLO 75: Preparación de Fusiones de Enzimas HA.

El cADN para la enzima de interés se puede aislar mediante una variedad de medios una incluyendo pero no exclusivamente a partir de bibliotecas de cADN, mediante RT-PCR y mediante PCR utilizando una serie de cebadores oligosintéticos de sobreposición, todos utilizando métodos estándares. El cADN se puede adaptar en los extremos 5' y 3' para generar sitios de restricción, tal que se pueden utilizar enlazadores □ubcutáneamente□, para clonar el cADN en un vector que contiene el cADN para HA. Esto puede ser en el término N o C con o sin el uso de una secuencia espaciadora. El cADN de enzima es clonado en un vector tal como pPPC0005 (Figura 2), sScCHSA, pScNHSA, o pC4:HSA a partir del cual el casete de expresión completo luego es cortado e insertado en el plásmido pSAAC35 para permitir la expresión de la proteína de fusión de albúmina en levadura. La proteína de fusión de albúmina secretada a partir de levadura luego puede ser recolectada y purificada a partir del medio y probada para su actividad biológica. Para expresión en líneas celulares mamíferas, un procedimiento celular es adoptado excepto que el casete de expresión utilizado emplea un promotor mamífero, secuencia guía y terminador (Ver Ejemplo 1). Luego este casete de expresión es cortado e insertado en un plásmido apropiado para la transfección de líneas celulares mamíferas.

EJEMPLO 76: Generación de Constructo ID 2053, IFNb-HSA.

El constructo ID 2053, pEE12.1:IFNb.HSA, comprende ADN que codifica una proteína de fusión de albúmina IFNb la cual tiene la proteína IFNb de longitud completa incluyendo la secuencia guía IFNb fusionada al amino-término de la forma madura de HSA en el vector de expresión NS0 pEE12.1.

Clonación de cADN de IFNb

El □ubcutáneament que codifica IFNb fue amplificado mediante PCR utilizando cebadores IFNb-1 y IFNb-2, descritos a continuación, cortados con *Bam* Hi/*Cla* I, y ligados en *Bam* Hi/*Cla* I cortados con pC4:HSA, resultando en constructo 2011. El fragmento *Eco* R1/*Eco* R1 del Constructo ID #2011 fue subclonado en el sitio *Eco* R1 de pEE12.1 generando el constructo ID #2053 el cual comprende ADN que codifica una proteína de fusión de albúmina que contiene la secuencia guía y la forma madura de IFNb, seguida por la proteína HSA madura.

Se sintetizaron dos □ubcutáneamente□ apropiados para amplificación PCR del □ubcutáneament que codifica la longitud completa de IFNb, IFNb-1 y IFNb-2:

IFNb-1:
5'GCGGGATCCGAATTCCGCCGCCATGACCAACAAGTGTCTCCTCCAAATTGCTCTCC TGTTGTGCTTCTCCACTACAGCTCTTTCCATGAGCTACAACTTGCTTGG-3'(SEQ ID NO:107)

IFNb-2:
5`-GCGCGCATCGATGAGCAACCTCACTCTTGTGTGCATCGTTTCGGAGGTAACCTGT-3'(SEQ ID NO: 108)

El cebador IFNb-1 incorpora un sitio de clonación *Bam* HI (mostrado subrayado), un sitio de clonación *Eco* RI, y una secuencia Kozak (mostrada en cursiva), seguido por 80 sanguínea que codifican los primeros 27 aminoácidos de la forma de longitud completa de IFNb. En IFNb-2, el

sitio *Cla* I (mostrado subrayado) y el ADN que sigue a este son el complemento inverso de ADN que codifica los 10 primeros aminoácidos de la proteína HSA madura (SEQ ID NO:1) y los últimos 18 sanguínea son el complemento inverso de ADN que codifica los últimos 6 residuos aminoácidos de IFNb (ver Ejemplo 2). Un amplimero PCR fue generado utilizando estos cebadores, purificado, digerido con enzimas de restricción *Bam* HI y *Cla* I, y clonado en los sitios *Bam* HI y *Cla* I del vector pC4:HSA. Después de confirmar la secuencia, un fragmento *Eco* RI conteniendo el casete de expresión de proteína de fusión de albúmina IFNb fue subclonado en pEEE12.1 digerido con *Eco* RI.

Además, el análisis del término-N de la proteína de fusión de albúmina mediante secuenciamiento de aminoácidos puede confirmar la presencia de la secuencia IFNb esperada (ver a continuación).

Las proteínas de fusión de albúmina de IFNb del invento preferiblemente comprenden la forma madura de HSA, es decir, Asp-25 a Leu-609, fusionada al extremo N o término C de la forma madura de IFNb, es decir, Met-22 a Asn-187. En una realización del invento, las proteínas de fusión de albúmina de IFNb del invento además comprenden una secuencia de señal la cual dirige el □ubcutáneam de fusión naciente en las rutas secretorias del □ubcutá utilizadas para expresión. En una realización preferida adicional, el péptido de señal codificado por la secuencia de señal es retirado, y la sanguínea de fusión de albúmina de IFNb madura es secretada directamente en el medio de cultivo. Las proteínas de fusión de albúmina de IFNb del invento pueden comprender las secuencas de señal heterólogas incluyendo, pero sin limitar a MAF, INV, Ig, Fibulina B, Clusterina, Proteína de Unión 4 de Factor de Crecimiento tipo Insulina, secuencias guía HSA variantes incluyendo, pero sin limitar a una secuencia guía HSA/MAF quimérica, u otras secuencias de señales heterologas conocidas en el estado de la técnica. En una realización preferida, las proteínas de fusión de albúmina de IFNb del invento comprenden el IFNb nativo. En realizaciones preferidas adicionales, las proteínas de fusión de albúmina de IFNb del invento comprenden además un residuo de metionina N-terminal. Los □ubcutáneamente que codifican estos polipéptidos, incluyendo fragmentos y/o variantes, también son contemplados por el invento.

Expresión y Purificación del Constructo ID 2053.

Expresión en lineas celulares NS0 de mieloma murino.

El constructo ID#2053, pEE12.1:IFNb-HSA, fue sometido a electroforesis en células NS0 mediante métodos conocidos en el estado de la técnica (ver Ejemplo 6).

Purificación a partir de sobrenadante de células NS0.

La purificación de IFNb-HSA a partir de sobrenadante de células NS0 pueden involucrar □ubcutáneamen de intercambio aniónico de Q-Sefarosa a pH 7.4 utilizando un gradiente de NaCl de 0 a 1 M en 20 mM Tris-HCl, seguido por □ubcutáneamen de intercambio aniónico Poros PI 50 a pH 6.5 con un gradiente de citrato de sodio de 5 a 4 mM, y diafiltrando para 6 DV en 10MM citrato, pH 6.5 y 140 mM NaCl, la composición de amortiguador final. El secuenciamiento N-terminal debe proporcionar la secuencia MSYNLL la cual es el término amino de la forma madura de IFNb. La proteína tiene un peso molecular aproximado de 88.5 kDa.

Para purificación a gran escala, ej., 50 L de sobrenadante de células NS0 se pueden concentrar en 8 a 10L aproximadamente. Luego la muestra concentrada puede ser pasada sobre columna de intercambio anionico de Q-Sefarosa (10 x 19 cm, 1.5 L) a pH 7.5 utilizando una □ubcutá por etapas constando de 50 mM NaOAc, pH 6.0 y 150 mM NaCl. Luego la muestra eluida puede ser inactivada viralmente con 0.75% Triton X 100 durante 60 min a temperatura ambiente. Luego se puede emplear □ubcutáneamen de fase inversa SDR (10 cm x 10 cm, 0.8 L) a pH 6.0 con 50 mM NaOAc y 150 mM NaCl, o alternativamente, la muestra se puede pasar

sobre una columna de SP-sefarosa a pH 4.8 utilizando una □ubcutá por etapas de 50 mM NaOAc, pH 6.0, y 150 mM HaCl. La filtración DV 50 seguiría para retirar cualquier contenido viral. Puede seguir □ubcutáneamen fenil-650M (20 cm x 12 cm, 3.8 L) a pH 6.0 utilizando una □ubcutá por etapas constando de 350 mM (NH4)2SO4 y 50 mM NaOAc, o alternativamente constando de 50 mM NaOAc pH 6.0. La diafiltración para 6-8 DV permitirá intercambio de amortiguador en el amortiguador de fórmulación final deseado de 10 mM Na2HPO4 + 58 mM sacarosa + 58 mM de sacarosa + 120 mM NaCl, pH 7.2 o 10 mM citrato, pH 6.5, y 140 mM NaCl o 25 mM Na2HPO4, 100 mM NaCl, pH 7.2.

La actividad de IFNb se puede evaluar utilizando un ensayo ISRE-SEAP in □ubcu.

Todas las proteínas de interferón tipo I señalan a través de un complejo receptor común y una ruta de señalización Jak/STAT similar que culmina en la activación de Interferón, "IFN", genes sensibles a través del Elemento Sensible de Sencuencia de Interferón, "ISRE". Un ensayo conveniente para actividad IFN tipo I es un sistema de ensayo basado en promotor-reportero que contiene múltiple copias del elemento ISRE fusionado a un gen reportero corriente abajo. Una línea celular HEK293 estable se puede generar y contiene una copia establemente integrada de un reportero ISRE-SEAP que es extremadamente sensible a IFNs tipo I y presenta linealidad sobre 5 registros de concentración.

Método de selección de clones estables.

El constructo 2053 fue sometido a electroforesis en células NS0 como se describió en el Ejemplo 6. Las células NS0 transfectadas con el constructo ID #2053 fueron seleccionadas para actividad al probar el medio de crecimiento acondicionado en el ensayo ISRE-SEAP. Las células receptores ISRE-SEAP/293F fueron sembradas en placas a 3 x 10 4 células/pozo en placas de 96 pozos recubiertas con poly-D-lisina, un día antes del tratamiento. Las células reporteras fueron tratadas con varias diluciones (incluyendo pero sin limitar a 1:500 y 1:5000) de sobrenadante acondicionado o preparaciones purificadas de proteína de fusión de albúmina IFNb codificada por el constructo ID 2053 o rhIFNb como un control. Luego las células reporteras fueron incubadas 24 horas antes de retirar 40 uL para utilizar en el Ensayo Quimioluminiscente de Genes Reporteros SEAP (catalogo Roche # 1779842). Se utilizo interferón humana recombinante beta, "rhIFNb" (Biogen), como un control positivo.

Resultado

La preparación purificada de IFNb-HSA expresada por NS0 tuvo un EC50 mayor de 9.3 x 10-9 g/mL que rhIFNb (Biogen) el cual tuvo un EC50 de 1.8 x 10-10 g/mL (ver Figura 4).

Inducción in vivo de OAS mediante una Interferón.

Método

La enzima OAS, 2'-5'- OligoAdenilato Sintasa, es activada a nivel transcripcional mediante interferón en respuesta a infección antiviral. El efecto de □ubcutáneam de interferón se puede medir al obtener muestras de sangre de monos tratados y analizando estas muestras para activación transcripcional de dos OAS mARN, p41 y p69. Un volumen de 0.5 mL de sangre entera se puede obtener a partir de cuatro animales por grupo en 7 puntos de tiempo diferentes, día 0, día 1, día 2, día 4, día 8, día 10, y día 14 por animal. Los diferentes grupos pueden incluir inyección de control de vehículo, inyección intravenosa y/o sanguínea de 30 ug/kg y/o 300 ug/kg de proteína de fusión de albúmina IFN el día 1, e inyección sanguínea de 40 ug/kg de interferón alfa (Schering-Plough) como control positivo los días 1, 3, y 5. Los niveles de transcriptos mARN p41 y p69 se pueden determinar mediante PCR □ubcutáneame de tiempo real (□ubcut) utilizando sondas específicas para p41-OAS y p69-OAS. Los niveles OAS mARN se pueden cuantificar con relación a control endógeno ARN ribosomal 18S.

Inducción in vivo de OAS mediante fusión de albúmina e interferón beta codificada por el constructo ID 2053.

Método

La actividad de la proteína de fusión HSA-IFNb codificada por el constructo 2053 se puede evaluar en el ensayo OAS *in vivo* como se describió anteriormente bajo el encabezado de sub-sección "inducción in vivo de OAS mediante una interferón".

EJEMPLO 77: indicaciones para proteínas de fusión de albúmina IFNb.

Las proteínas de fusión de albúmina IFN beta (incluyendo, pero sin limitar a aquellas codificadas por el constructo 2053) se puede utilizar para tratar, prevenir, aliviar de y/o detectar esclerosis múltiple. Otras indicaciones incluyen, pero no están limitadas a infecciones virales incluyendo síndrome respiratorio agudo severo (SARS) y otras infecciones por coronavirus; filovirus, incluyendo pero sin limitar a virus Ebola y virus Marburg; Arenavirus, incluyendo pero sin limitar a virus Pichende, virus Lassa, virus Machupo, virus Guanarito; y virus de coriomeningitis linfocítico (LCMV); Bunyavirus, incluyendo pero sin limitar a virus Punta toro, virus de fiebre hemorrágica Crimean-Congo, virus de fiebre de mosca de arena, virus de fiebre del Valle de Rift, virus La crosse, y hantavirus; flavivirus, incluyendo pero sin limitar a fiebre amarilla, virus Banzi, virus del Nilo occidental, virus de dengue, virus de Encefalitis Japonesa, encefalitis transmitida por garrapata, fiebre hemorrágica Omsk, y virus de Enfermedad Forestal de Kyasanur; Togavirus, incluyendo pero sin limitar a virus de encefalitis equina venezolana, oriental y occidental, virus de Ross River; Orthopoxvirus, incluyendo pero sin liminar Vaccinia, viruela vacuna, viruela, y viruela de los monos; virus del herpes; FluA/B; virus sincitial respiratorio (RSV); sarampión; rinovirus; adenovirus; virus Semliki Forest; fiebres hemorrágicas virales; rabdovirus; paramixovirus, incluyendo pero sin limitar a Nipah virus y virus Hendra; y otros agentes virales identificados por los centros para control y prevención de enfermedades como agentes patológicos de alta prioridad (es decir, agentes Categoría A, B y C; ver, *ej.*, Moran, Emerg, Med. Clin. North. Am., 2002; 20(2):311-30 y Darling, Emerg. Med. Clin. North. Am. 2002; 20(2):273-309).

EJEMPLO 78: Generación de Constructo ID 2249, IFNa2-HSA.

El constructo ID 2249, pSAC35:IFNa2.HSA, comprende ADN que codifica una proteína de fusión de albúmina IFNa2 la cual tiene la secuencia guía quimérica HSA, seguida por la forma madura de la proteína IFNa2, es decir, fusionada al extremo amino de la forma madura de HSA en el vector de expresión *S. cerevisiae* pSAC35 de levadura.

Clonación de cADN de IFNa2

El □ubcutáneament que codifica IFNa2 fue amplificado utilizando los cebadores IFNa2-1 y IFNa2-2, descritos a continuación. El amplimero PCR fue cortado con *Sal* I/Cla I, y ligado en pSCCHSA cortado por *Xho I*/Cla I. El constructo ID #2249 codifica una proteína de fusión de albúmina que contiene la secuencia guía quimérica de HSA, la forma madura de IFNa2, seguida por la proteína HSA madura.

Dos □ubcutáneamente□ apropiados para amplificación PCR del □ubcutáneament que codifica la forma madura de IFNa2, IFNa2-1, y IFNa2-2, fueron sintetizados:

IFNa2-1:

5`-CGCGCGCGTCGACAAAAGATGTGATCTGCCTCAAACCCACA-3`(SEQ ID NO:109)

IFNa2-2:

5`-

GCGCGCATCGATGAGCAACCTCACTCTTGTGTGCATCTTCCTTACTTCTTAAACTTTC T-3′ (SEQ ID NO:110)

El cebador IFNa2-1 incorpora un sitio de clonación *Sal* 1 (mostrado subrayado), sanguínea que codifican los últimos tres residuos aminoácidos de la secuencia guía HSA quimérica, así como también 22 sanguínea (mostrados en negrilla) que codifican los primeros siete residuos aminoácidos de la forma madura de IFNa2. En IFNa2-2, el sitio *Cla* 1 (mostrado subrayado) y el ADN que sigue son el complemento inverso de ADN que codifica los primeros 10 aminoácidos de la proteína HSA madura y los últimos 22 sanguínea (mostrados en negrilla) son el complemento inverso de ADN que codifica los últimos siete residuos aminoácidos de IFNa2 (ver Ejemplo 2). Un amplimero PCR de IFNa2-HSA fue generado utilizando estos cebadores, purificado, digerido con enzimas de restricción *Sal* 1 y *Cla* I, y clonado en los sitios *Xho* I y *Cla* del vector pScCHSA. Después de confirmar la secuencia, el casete de expresión que codifica esta proteína de fusión de albúmina IFNa2 fue subclonada en pSAC35 digerida por *Not.*

Además, el análisis del término N de la proteína de fusión de albúmina expresada por el secuenciamiento aminoácido puede confirmar la presencia de la secuencia IFNa2 esperada (ver más adelante).

Otras proteínas de fusión de albúmina IFNa2 que utilizan diferentes secuencias guía han sido construidas mediante métodos conocidos en el estado de la técnica (ver Ejemplo 2). Ejemplos de varias secuencias guía incluyen, pero no están limitadas a, invertasa "INV" (□ubcutáneam 2343 y 2410) y factor alfa de acoplamiento "MAF" (constructo 2366). Estas proteínas de fusión de albúmina IFNa2 se pueden subclonar en vectores de expresión mamíferos tales como pC4 (□ubcutáneam 2382) y pEE12.1 como se describió previamente (ver Ejemplo 5). También se pueden construir proteínas de fusión de albúmina IFNa2 con el término C fusionado de porción terapéutica a HSA(constructo 2381).

Las proteínas de fusión de albúmina IFNa2 del invento preferiblemente comprenden la forma madura de HSA, es decir, Asp-25 a Leu-609, fusionada al extremo N o C de la forma madura de IFNa2, es decir Cys-1 a Glu-165. En una realización del invento, las proteínas de fusión de albúmina IFNa2 del invento además comprenden una secuencia de señal que dirige el □ubcutáneam de fusión naciente en las rutas secretorias del huésped utilizado para expresión.

En una realización adicional preferida, el péptido de señal codificado por la secuencia de señal es retirado, y la proteína de fusión de albúmina IFNa2 madura es secretada directamente en medio de cultivo. Las proteínas de fusión de albúmina IFNa2 del invento pueden comprender secuencias de señal heterólogas incluyendo, pero sin limitar a, MAF, INV, Ig, Fibulina B, Proteína 4 de Unión de Factor de Crecimiento tipo Insulina, secuencias guía de HSA variante incluyendo, pero sin limitar a, una secuencia guía HSA/MAF quimérica, u otras secuencias de señal heterólogas conocidas en el estado de la técnica. En una realización preferida, las proteínas de fusión de albúmina IFNa2 del invento comprenden la IFNa2 nativa. En realizaciones preferidas adicionales, las proteínas de fusión de albúmina IFNa2 del invento comprenden además un residuo de metionina N-terminal. Los □ubcutáneamente que codifican estos péptidos, incluyendo fragmentos y/o variantes, también son contemplados por el invento.

Expresión y purificación de constructo ID 2249.

Expresión en levadura S. carevisiae.

La transformación del constructo 2249 en levadura *S. cerevisiae* cepa BXP10 fue realizada mediante métodos conocidos en el estado técnica (ver Ejemplo 3). Las células pueden ser recolectadas en la fase estacionaría después de 72 horas de crecimiento. Los sobrenadantes son recolectados al clarificar las células a 3000g durante 10 min. Los niveles de expresión son examinados mediante detección inmunosorbente con suero anti-HSA (Kent □ubcutáneame) o como el anticuerpo primario. La proteína de fusión de albúmina IFNa2 de peso molecular aproximado de 88.5 kDa puede ser obtenida.

Purificación a partir de sobrenadante celular de levadura S. cerevisiae

El sobrenadante celular que contiene proteína de fusión de albúmina IFNa2 expresado desde el constructo ID #2249 en células de levadura *S. cerevisiae* puede ser purificado a escala pequeña sobre una columna de afinidad □ubcután̈e Dyax (ver Ejemplo 4) o a gran escala siguiendo 5 pasos: diafiltración, cromatografía de intercambio aniónico utilizando columna de Flujo Sanguínea de DEAE-Sefarosa, cromatografía de interacción hidrofóbica (HIC) utilizando columna de butilo 659S, cromatografía de intercambio catiónico utilizando una columna de Flujo Rápido de SP-Sefarosa o una □ubcutáneamen de Azul-Sefarosa, y cromatografía de alto desempeño que utiliza cromatografía de columna de alto desempeño Q-sefarosa (ver Ejemplo 4). La proteína de fusión de albúmina IFNa2 puede eluir a partir de la columna de Flujo Rápido de DEAE-Sefarosa con 100 – 250 mM NaCl, a partir de la columna de flujo rápido de SP-Sefarosa con 150 – 250 mM NaCl, y a partir de la columna de alto desempeño de Q-sefarosa a 5 – 7.5 mS/cm. El secuenciamiento N-terminal debe proporcionar la secuencia CDLPQ (SEQ ID NO:98) la cual corresponde a la forma madura de IFNa2.

La actividad de IFNa2 se puede evaluar utilizando en ensayo ISRE-SEAP in □ubcu.

Método

La proteína de fusión de albúmina codificada por el constructo ID # 2249 puede ser probada para actividad en el ensayo ISRE-SEAP como se describió anteriormente en el ejemplo 76. Brevemente, los sobrenadantes de levadura acondicionados fueron probados a una dilución 1:1000 para su capacidad para dirigir la transducción de señales ISRE sobre la línea celular reportera ISRE-SEAP/293F. Las células reporteras ISRE-SEAP/293F fueron sembradas en placas a 3 x 10 4 células/pozo en placas de 96 pozos recubiertas con poli-D-lisina, un día antes del tratamiento. Luego las células reporteras fueron incubadas durante 18 o 24 horas antes de retirar 40 uL para utilizar en el Ensayo Quimioluminiscente del Gen Reportero SEAP (# de Catalogo Roche 1779842). Interferón humana recombinante beta, "rhIFNb" (Biogen), se utilizo como un control positivo.

Resultado

La preparación purificada de IFNa2-HSA demostró un aumento relativamente lineal sobre concentraciones oscilando entre 10-1 a 10 1 ng/mL (ver Figura 5) o 10-10 a 10-8 ng/mL (ver Figura 6).

Inducción in vivo de OAS mediante fusión de Interferón alfa codificada por el constructo ID 2249

Método

La enzima OAS, 2'-5' OligoAdenilato Sintasa, es activada a nivel transcripcional mediante la interferón en respuesta a infección bacteriana. El efecto de □ubcutáneam de interferón se puede medir al obtener muestras de sangre de monos tratados y analizar estas muestras para activación transcripcional de dos OAS mARN, p41 y p69. Un volumen de 0.5 mL de sangre entera fue obtenido de 4 animales por grupo en 7 puntos de tiempo diferentes, día 0, día 1, día 2, día 4, día 8, día 10, y día 14 por animal. Los diferentes grupos pueden incluir control de vehículo, inyección intravenosa de 30 ug/kg HSA-IFN el día 1, inyección sanguínea de 30 ug/kg de HSA-IFN el día 1, inyección sanguínea de 300 ug/kg de HSA-IFN el día 1, e inyección sanguínea de 40 ug/kg de interferón alfa (Schering-Plough) como un control positivo los días 1, 3 y 5. Los niveles de transcriptos mARN p41 y p69 fueron determinados mediante PCR □ubcutáneame de tiempo real (□ubcut) utilizando sondas específicas para p41-OAS y p69-OAS. Los niveles OAS mARN se pueden cuantificar con relación a control endógeno ARN ribosomal 18S. La fusión de albúmina codificada por el constructo 2249 se puede someter a experimentación similar.

Resultados

Un aumento significativo en niveles de transcriptos mARN para ambos p41 y p69 OAS se observó en monos tratados con HSA-interferón en contraste con monos tratados con IFNa (ver Figura 7 para datos p41). El efecto duro casi 10 días.

EJEMPLO 79: Indicaciones para proteínas de fusión de albúmina IFNa2.

Las proteínas de fusión de albúmina IFN alfa (incluyendo, pero sin limitar a aquellas codificadas por los □ubcutáneam 2249, 2343, 2410, 2366, 2382, y 2381) se pueden utilizar para tratar, prevenir, aliviar, y/o detectar esclerosis múltiple. Otras indicaciones incluyen, pero no están limitadas e infecciones virales incluyendo síndrome respiratorio agudo severo (SARS) y otras infecciones por coronavirus; filovirus, incluyendo pero sin limitar a virus Ebola y virus Marburg; Arenavirus, incluyendo pero sin limitar a virus Pichende, virus Lassa, virus Junin, virus Machupo, virus Guanarito; y virus de coriomeningitis linfocítico (LCMV); Bunyavirus, incluyendo pero sin limitar a virus Punta toro, virus de fiebre hemorrágica Crimean-Congo, virus de fiebre de mosca de arena, virus de fiebre del Valle de Rift, virus La crosse, y hantavirus; flavivirus, incluyendo pero sin limitar a fiebre amarilla, virus Banzi, virus del Nilo occidental, virus de dengue, virus de Encefalitis Japonesa, encefalitis transmitida por garrapata, fiebre hemorrágica Omsk, y virus de Enfermedad Forestal de Kyasanur; Togavirus, incluyendo pero sin limitar a virus de encefalitis equina venezolana, oriental y occidental, virus de Ross River; Orthopoxvirus, incluyendo pero sin liminar Vaccinia, viruela vacuna, viruela, y viruela de los monos; virus del herpes; FluA/B; virus sincitial respiratorio (RSV); sarampión; rinovirus; adenovirus; virus Semliki Forest; fiebres hemorrágicas virales; rabdovirus; paramixovirus, incluyendo pero sin limitar a Nipah virus y virus Hendra; y otros agentes virales identificados por los centros para control y prevención de enfermedades como agentes patológicos de alta prioridad (es decir, agentes Categoría A, B y C; ver, *ej.*, Moran, Emerg, Med. Clin. North. Am., 2002; 20(2):311-30 y Darling, Emerg. Med. Clin. North. Am. 2002; 20(2):273-309).

Preferiblemente, la proteína de fusión de albúmina IFN-alfa o proteína de fusión híbrida IFN se administra en combinación con un antagonista CCR5, además en asociación con al menos una entre ribavirina, IL-2, IL-12, pentasufida sola o en combinación con una terapia farmacológica anti-VIH, ej., HAART, para la preparación de un medicamente para el tratamiento de infecciones de VIH-1, VHC, o co-infecciones de VIH-1 y VHC en pacientes pedriaticos y adultos.

Ejemplo 80: Generación de Constructo ID #3691, BNP-HSA.

El constructo ID # 3691, pC4:SPCON.BNP1-32/HSA, comprende ADN que codifica un proteína de fusión de albúmina BNP la cual tiene una secuencia sanguínea de consenso, seguida por el péptido BNP activo procesado (aminoácidos 1-32) fusionado al extremo amino de la forma madura de HSA en el vector de expresión mamífera pC4.

Clonación de cADN de BNP para el constructo 3691

El □ubcutáneament que codifica BNP fue amplificado por PCR utilizando los cebadores BNP-1 y BNP-2, descritos a continuación, cortados con *Bam* HI/*Cla* 1, ligados en pC4:HSA cortado con *Bam HI/Cla* resultando en el constructo ID #3691. El constructo ID # 3691 codifica una proteína de fusión de albúmina que contiene una secuencia guía de consenso (SEQ ID No:111) y la forma activa procesada de BNP, seguida por la proteína Hsa madura (ver SEQ ID No: 321 para el constructo 3691 en la Tabla 2).

Dos □ubcutáneamente□ apropiados para amplificación PCR del □ubcutáneament que codifica la forma activa procesada de BNP, BNP-1 y BNP-2, fueron sintetizados:

BNP-1:

5`-
GAGCGCGGATCCAAGCTTCCGCCATCATGTGGTGGCGCCTGTGGTGGCTGCTGCTGC
TGCTGCTGCTGCTGTGGCCCATGGTGTGGGCCAGCCCCAAGCTGGTGCAAGG -3´
(SEQ ID NO:463)

BNP-2:
5`-
AGTCCCATCGATGAGCAACCTCACTCTTGTGTGCATCATGCCGCCTCAGCACTTTGC-
3`(SE Q ID NO: 464).

BNP-1 incorpora un sitio de clonación *Bam* H1 (subrayado), □ubcutáneamente que codifican una secuencia guía de consenso (SEQ ID No:111) (cursiva), y □ubcutáneamente que codifican las primeras siete □ubcutánea □ubcutáneam de BNP (negrilla). En BNP-2, la secuencia subrayada es un sitio *Cla* I, y los □ubcutáneamente que la siguen contienen el complemente inverso de ADN que codifica los últimos 6 aminoácidos de BNP (negrilla) y los primeros 10 aminoácidos de la proteína madura HSA. Utilizando estos dos cebadores la proteína BNP fue amplificada mediante PCR. Las temperaturas y tiempos de recocido y extensión se deben determinar □ubcutáneamen para cada plantilla y par de cebadores sanguínea.

El producto PCR fue purificado (por ejemplo, utilizando el Sistema de Purificación de ADN Prep PCR Wizard (Promega Corp)) y luego digerido con *Bam* HI y *Cla* I. Después de purificación adicional del fragmento *Bam* HI-*Cla* mediante electroforesis de gel, el producto fue clonado a pC4:HSA digerido con *Bam* HI/*Cla* I para producir el constructo ID # 3691. El constructo de expresión fue verificado por secuencia.

Expresión y Purificación de Constructo ID 3691

Expresión de células 293F

El constructo ID # 3691, pC4:SPCON.BNPI-32/HSA, fue transfectado a células 293F mediante métodos conocidos en el estado de la técnica (ver Ejemplo 6).

Purificación a partir de sobrenadante de células 293F.

Dos litros de sobrenadante fueron recolectados 3 días después de la transfección. La proteína recombinante fue capturada mediante 5 ml de columna CL-6B de Sefarosa Azul (Amershan Biosciences, Piscataway, NJ, USA) y y eluida mediante 2 M NaCl. El material se unió a columna Hiprep 16/10 Fenil FF (hig sub) y fue eluido mediante 20 mM MES, pH 6.7. BNP-HSA se purificó adicionalmente mediante □ubcutáneamen en columna de hidroxiapatita en gradiente de amortiguador de fosfato de sodio (0-20 mS/cm en 200 ml) a pH 6.8. El producto final fue intercambiado en PBS pH 7.2 mediante una columna HiPrep 26/10 desalting (Amersham Biosciences).

La actividad de BNP-HSA se puede evaluar utilizando un ensayo in □ubcu NPR-A/cGMP.

El receptor-A sanguínea natriurético (NPR-A) es el receptor de señalización para BNP, y como tal, es responsable de la mayoría de efectos biológicos de BNP. La bioactividad BNP es mediada por el dominio guanililo ciclasa NPR-A GTP a cGMP ante activación. Un ensayo conveniente para actividad BNP es medir la estimulación BNP de una línea celular 293F que establemente sobreexpresa NPR-A. La producción cGMP en las células después de exposición a BNP se pueden medir mediante cGMP ELISA.

Método de Selección de clones estables de NPR-A 293F.

El marco de lectura abierta de NPR-A humana se construyo en el vector de expresión pcADN3.1 (Invitrogen). Las células 293F fueron establemente transfectadas con el ADN plásmido mediante el método de Lipofectamina y seleccionadas mediante 0.8 ug/ml de G418.

Los clones estables 293F/NPR-A fueron seleccionados para la mejor respuesta a BNP recombinante.

Medición de activación cGMP.

La activación cGMP por BNP se realizo en células 293F/NPR-A y se midió mediante un kit de ensayo fluorescente GMP cíclico CatchPoint (Molecular Devices, Sunnyvale, CA, USA). Brevemente, 50.000 células/pozo de células 293F/NPR-A cultivadas en una placa de 96 pozos fueron lavadas en 80 ul de amortiguador de pre-estimulación (amortiguador de bicarbonato Krebs-Ringer con 10 mM de glucosa, pH 7.4, 15 nM bicarbonato de sodio, y 0.75 mM 3-isobutil-1-metilxantina). Se agregó BNP-HSA o BNP recombinante en 40 ul de amortiguador de □ubcutáneamente a las células a 37°C durante 10 minutos. Las células fueron sometidas a lisis con 40 ul de amortiguador de lisis durante 10 min con agitación. Las cantidades de cGMP en los lisados fueron cuantificadas según instrucción del fabricante.

Resultado

La relación dosis-respusta de BNP-HSA y BNP recombinante fue determinada (ver Figura 8). Las actividades máximas del Constructo ID # 3691 y BNP recombinante furon similares (1.63 +- 0.016 vs. 1.80 +- 0.016 pm, respectivamente), con valores EC50 de 28.4 +- 1.2, y 0.46 +- 1.1 nM, respectivamente.

BNP-HSA disminuye presión sanguínea in vivo.

BNP reduce la presión sanguínea mediante vasodilación directa así como también mediante eliminación de sistemas de renina/angiotensina/endotelina/aldosterona. La capacidad de BNP-HSA para disminuir presión sanguine arterial fue probada en ratas espontáneamente hipertensas macho de tres meses de edad compradas en Taconic (Germantown, NY, USA). Las ratas espontáneamente hipertensas son genéticamente hipertensas con comienzo de presión sanguínea alta después de tres meses de edad. BNP-HSA o BNP recombinante fue reconstitudo en 0.3 cc PBS por rata. Los fármacos fueron liberados a través de inyección en la vena de la cola. Las presiones sistólica y diastólica fueron registradas mediante el método muñon-cola utilizando el Sistema XBP-1000 (Kent Scientific, Torrigton, CT, USA). Para cada punto de datos de presión sanguínea, se tomaron y promediaron 4-5 lecturas consecutivas. La presión arterial media (MAP) fue calculada como 1/3 de presión sistólica + 2/3 de presión diastólica. Para determinación dosis-respuesta, las presiones sanguineas fueron medidas 20 h después de administración de pC4:SPCON.BNP1-32/HSA a dosis de 0.5, 2, 6, y 18 nmol/kg.

Resultado

La presión sistólica típica de ratas espontáneamente hipertensas fue 180-200 mmHg antes de dosificación. Un bolo simple de 6 nmol/kg de BNP-HSA liberado a través de inyección intravenosa redujo tanto la presión sistólica como diastólica, y fue responsable de reducción de más de 30 mmHg de presión arterial media (MAP). La presión arterial reducida fue estable y continuó durante un día y luego gradualmente retornó a la línea base durante varios días (ver Figura 9). En contraste, debido a su eliminación instantanea, un bolo simple de 6 nmol/kg de BNP recombinante, produjo una disminución MAP muy temporal de aproximadamente 15 mmHg.

Además, la respuesta de dosis 20 horas después de la inyección de un bolo de BNP-HSA se determinó en cuatro ratas espontáneamente hipertensas. 0.5 nmol/kg BNP-HSA tuvo un promedio de reducción MAP de 7 mmHg, mientras 6 nmol/kg de BNP-HSA tuvo un promedio de reducción de 30 mmHg MAP, y una dosis alta de 18 nmol/kg de BNP-HSA solo redujo la presión sanguínea levemente más de 6 nmol/kg.

Inducción in vivo de cGMP de plasma mediante BNP-HSA.

Método

La activación de cGMP intracelular mediante BNP resulta en su liberación desde la célula a circulación. El nivel de cGMP de plasma está en correlación con fisiología renal y cardiovascular BNP inducida. cGMP de plasma se ha utilizado como un biomarcador para acción BNP *in vivo*. Para probar la inducción de cGMP de plasma mediante BNP-HSA *in vivo*, ratones C57/BL6 macho de 11 a 12 semanas de edad recibieron un bolo simple de BNP o BNP-HSA recombinante a una dosis de 6 nmol/kg a través de la vena de la cola. El plasma se preparó a partir de sangrados de la cola en puntos de tiempos de 5, 10, 20, 40, y 80 min para el grupo de dosificación de BNP recombinante y a 640, 1440, 2880 y 5760 minutos adicionales para el grupo BNP-HSA. Las muestras de plasma de ratones tratados con PBS como el control de vehículo fueron recolectadas como puntos de tiempo cerco. Los niveles cGMP fueron determinados mediante un kit de ensayo fluorescente de GMP cílico CatchPoint según instrucción del fabricante.

Resultado

Después de un bolo simple intravenoso de 6 nmol/kg de BNP o BNP-HSA recombinante, los niveles pico de cGMP de plasma sobre la base fueron aumentados 3.9 o 5.6 veces, respectivamente (ver Figura 10). Además, la vida media de disminución exponencial de una fase de cGMP después de tratamiento BNP recombinante fue 16 min (10 a 42 min, 95%CI), mientras la vida media de disminución exponencial de una fase de cGMP después de administración BNP-HSA fue 1538 min (1017 a 3153 min, 95%CI).

Análisis farmacocinético in vivo de fusión de albúmina BNP codificada por el constructo ID 3691.

Método

Ratones macho de 11 a 12 semanas de edad C57/BL6 (obtenidos en Ace Animals, Boyertown, PA, USA) con peso de 25.1 +- 0.12 g en el momento del estudio. Todos los animales fueron dosificados a un volumen de 10 ml/kg de peso corporal. Los animales de predosis fueron inyectados con PBS. Se inyecto BNP recombinante intravenosamente en la cola o subcutáneamente en la región escapular media.

El análisis fármacocinético se realizó sobre los siguientes grupos:

Tabla 8

Grupo	Fármaco	Dosis(mg/kg)	Ruta	N/Tiempo	Tiempo (horas)
1	BNP-HSA	2.19	Subcutánea	3	0.5, 2, 6, 16, 24, 32, 48, 72, 96
2	BNP-HSA	2.19	Intravenosa	3	0.083, 2, 6, 16, 24, 32, 48, 72, 96
3	Vehículo	0	Subcutánea	3	Predosis
4	Vehículo	0	Intravenosa	3	predosis

Se recolectó muestra de sangre de la vena cava inferior, se colocó en un microtainer recubierto con EDTA, y se almacenó sobre hielo. Las muestras fueron configuradas en una microcentrifuga a 14.000 rpm (16.000 x g) durante 10 minutos a temperatura ambiente. El plasma fue transferido a tubos cluster y almacenado a -80°C.

Las concentraciones de BNP-HSA en muestras de plasma fueron determinadas utilizando el kit BNP EIA (Phoenix Pharmaceutical, Belmont, CA, USA). Las curvas estándares fueron generadas al mismo tiempo sobre la misma placa con muestras de prueba. El límite de detección fue 0.11 ng/mL para BNP recombinante. El ensayo detecta BNP recombnante y no reacciona de manera cruzada a BNP de ratón.

El análisis fue realizado mediante métodos no comparmentales (WinNonlin; versión 4.1; Pharsight Corp, Mountain Viwe, CA, USA). La concentración media de plasma en cada tiempo fue utilizada en el análisis. Un método trapezoidal lineal se utilizó para calcular AUC0-t. La extrapolación a infinidad AUC0 se realizo al dividir la última concentración observada por la constante de tasa de elimnación termial. Los datos fueron uniformemente sopesados para tres análisis.

Resultado

La concentración base media de BNP-HSA en plasma como se detectó en las muestras pre-dosis fue aproximadamente 0.081-0.095 ug/ml. Después de una única inyección intravenosa o subcutanea, BNP-HSA tuvo vidas medias de eliminación terminal de 11.2 (liberación intravenosa) o 19.3 h (liberación subcutanea), mientras la vida media de BNP recombinante en ratones fue 3.1 min. El análisis no compartmental de BNP-HSA reveló que BNP-HSA tuvo las siguientes características:

Tabla 9

	Unidad	Intravenosa	Subcutánea
T_{max}	H	NA	16
C_{max}	µg/ml	NA	11.2
$T_{1/2,term}$	h	11.2	19.3
AUC_{0-}	(h. µg/ml)(m/kg)	658.9	227.9
V_{ss}	Ml/Kg	37	NA
V_z o V_z/F	Ml/kg	53.5	268
CL o CL/F	Ml/kg	3.3	9.6
MRT	H	11.2	19.8
Bioavilidad	%		34.6

Cinco puntos en la fase terminal del perfil intravenoso y cuatro puntos en la fase terminal del perfil subcutaneo fueron seleccionados para cálculo de vida media términal. La AUC resultante durante esta fase terminal fue aproximadamente 10% de la AUC total para los perfiles intravenoso y subcuteneo, respectivamente. Esto se compara a solamente 2% y 4% de la AUC total para el perfil intravenoso y subcutaneo, respectivamente, cuando los últimos tres puntos fueron seleccionados para el calculo de vida media terminal.

EJEMPLO 81: Generación de Constructo ID # 3618, BNP(2X).

El constructo ID # 3618, pC4:SPCON.BNP-32(2x)/HSA, comprende ADN que codifica una proteína de fusión de albúmina BNP la cual tiene una secuencia guía de consenso, secreton, seguida por dos péptidos BNP activos (aminoácidos 1-32) en tandem fusionados con el extremo amino de la forma madura de HSA en el vector de expresión mamífero pC4.

Clonación de cADN de BNP para el constructo 3618

El polipéptido que codifica la fracción BNP duplicada primero fue PCR amplificado a partir a partir de la forma activa procesada de BNP (aminoácidos 1-32) utilizando cuatro cebadores BNP-1, BNP-2, y BNP-4, descritos más adelante, para crear dos fragmentos A y B. Después de amplificación, los dos fragmentos purificados (A y B) fueron mezclados en una

cantidad molar igual y se utilizaron como un PCR plantilla y se amplificaron con los cebadores BNP-5 y BNP-6, como se describe a continuación. El inserto BNP(2x) luego fue digerido con *Bam*HI y *ClaI y* ligado en el vector pC4HSA pre-digerido con *Bam*HI y *Cla*I resultando en el constructo 3618. El constructo ID # 3818 codifica una proteína de fusión de albúmina que contiene una secuencia guía de consenso, secreton (SEQ ID No. 111), y dos copias en tándem de la forma activa procesada de BNP, seguida por la proteína HSA madura (ver SEQ ID No. 483 para el constructo 3618 en la Tabla 2).

Primero se sintetizaron cuatro oligonucleotidos apropiados para amplificación PCR de los polinucleotidos que codifican dos fragmentos de la proteína BNP:

BNP-1

5ÀGCCCCAAGATGGTGCAAGGGTCTGGCTGCTTTGGGAGGAAGATGGACCGG ATCAGCTCCTCCAGTGGCTGGGCTGCAAAGTGCTGAGGCGGCAT-3`(SEQ ID NO:486)

BNP-2 5`-CCTTGCACCATCTTGGGGCTATGCCGCCTCAGCACTTTGC-3`(SEQ ID NO:487)

BNP-3 5``GCAAAGTGCTGAGGCGGCATAGCCCCCAAGATGGTGCAAGG-3`(SEQ ID NO:488)

BNP-4 5`-AGTCCCATCGATGAGGCGGCATAGCCCCAAGATGGTTGCAAGG-3`(SEQ ID NO:488)

Utilizando grupos de cebadores BNP-1/BNP-2 y BNP-3/BNP-4, dos fragmentos de proteínas BNP (A y B, respectivamente) fueron amplificados mediante PCR. Las temperaturas y tiempos de recocido y extensión se determinaron empiricamente para par especifico de cebador y plantilla. Los fragmentos A y B fueron purificados (por ejemplo, utilizando el Sistema de Purificación de ADN Preps PCR Wizard (Promega Corp)), mezclados en cantidades molares iguales, y utilizados como una plantilla para amplificación PCR utilizando dos oligonucleotidos apropiados para amplificación PCR, BNP-5 y BNP-6:

BNP-5: 5`-
GAGCGCGGATCCAAGCTTCCGCCATCATGTGGTGGCGCCTGTGGGTGGCTGCTGCTG CTGCTTGCTGCCTGCTGTGGCCCATGGTGTGGGCCAGCCCCAAGCTGGTGCAAGG-3′(SEQ ID NO:463)

BNP-6: 5′-
AGTCCCATCGATGAGCAACCTCACTCTTGTGTGCATCATGCCGCCTCAGCACTTTGC-3`(SEQ ID MO:464)

BNP-5 incorpora un sitio de clonación *Bam* HI (subrayado), polinucleotidos que codifican una secuencia guía de consenso (SEQ ID No. 111) (cursiva), y polinucleotidos que codifican las primeras siete secuencias de aminoácidos de BNP (negrilla). En BNP-6, la secuencia subrayada es un sitio *Cla* I, y los polinucleotidos que la siguen contienen el complemento inverso de ADN que codifica los últimos 6 aminoácidos de BNP (negrilla) y los primeros 10 aminoácidos de la proteína HSA madura. Utilizando estos dos cebadores, una secuencia guía de consenso y dos copias en tándem de peptidos BNP activos fueron amplificadas mediante PCR. Las temperaturas y tiempos de cocimiento y extensión se deben determinar empiricamente para cada par específico de cebadores y plantilla.

El producto PCR se purificó (por ejemplo, utilizando el Sistema de Purificación de ADN Wizard PCR Preps (Promega Corp)) y luego fue digerido con *Bam* HI y *Cla* I. Después de purificación adicional del fragmento *Bam* HI-*Cla* 1 mediante electroforesis de gel, el producto fue clonado a *Bam* HI/*Cla* I digerido pC4:HSA para producir el constructo ID # 3618. El constructo de expresión fue verificado por secuencia.

Expresión y Purificación del Constructo ID #3618

Expresión en células 293F

El constructo ID # 3618, pC4:SPCON.BNP1-32(2x)/HSA fue transfectado a células 293F mediante metodos conocidos en el estado de la técnica (ver Ejemplo 6).g

Purificación a partir de sobrenadante de células 293F.

pC4:SPCON.BNP1-32(2x)/HSA codificada mediante el Constructo ID # 3618 fue purificado como se describió anteriormente en el Ejemplo 80 bajo el encabezado de subsección "Purificación a partir de sobrenadante de células 203F."

La actividad de BNP(2x)-HSA se puede evaluar utilizando un ensayo in vitro de NPR-A/cGMP.

La actividad de BNP(2x)-HSA codificada mediante el constructo ID #3618 se puede evaluar *in vitro* utilizando un ensayo NPR-A/cGMP como se describió anteriormente en el Ejemplo 80 bajo el encabezado de subsección. "La actividad de BNP-HSA se puede evaluar utilizando un ensayo in vitro NPR-A/cGMP," y "Método de Clasificación de Clones Estables NPR-A 203F."

Resultado

La relación dosis-respuesta de BNP(2x)-HSA y BNP recombinante fue determinada (ver Figura 8). Las actividades máximas de BNP(2X)-HSA codificadas por el Constructo ID # 3618, y BNP recombinante fueron similares (1.68 +- 0.021, vs 1.80 +- 0,.016 pm, respectivamente) con valores EC50 de 9.8 +- 1.1 y 0.46 +- 1.1 nM respectivamente.

La divulgación completa de cada documento citado (incluyendo patentes, solicitudes de patentes, publicaciones de patentes, articulos de diario, resumenes, manuales de laboratorio, libros, u otras divulgaciones) así como también información disponible a través de identificadores específicos a bases de datos tales como GenBank, GeneSeq, o el registro CAS, citados en esta solicitud son incorporados en la presente como referencia en su totalidad.

Además, la especificación y listado de secuencias de cada una de las siguientes solicitudes americanas son incorporadas como referencia en su totalidad: Solicitud Americana No. 60/542.274, registrada en Febrero 4 de 2005; solicitud americana No. 60/549.901, registrada en marzo 5 de 2004; solicitud americana No. 60/556.906, registrada en marzo 29 de 2004; y solicitud americana No. 60/636.603, registrada en diciembre 17 de 2004.

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REIVINDICACIONES

1. Una proteína de fusión de albúmina que comprende un miembro seleccionado del grupo que consta de:

(a) una proteína terapéutica: X y albúmina que comprende la secuencia de aminoácidos de SEQ ID NO:1;

(b) una proteína terapéutica: X y un fragmento o una variante de la secuencia de aminoácidos de SEQ ID NO:1, en donde dicho fragmento o variante tiene actividad de albúmina;

(c) una proteína terapéutica: X y un fragmento o una variante de la secuencia de aminoácidos de SEQ ID NO: 1, en donde dicho fragmento o variante tiene actividad de albúmina, y en donde además dicha actividad de albúmina es la capacidad para prolongar la vida en almacenamiento de la proteína terapéutica: X en comparación con la vida en almacenamiento de la proteína terapéutica: X en un estado sin fusionar;

(d) una proteína terapéutica: X y un fragmento o una variante de la secuencia de aminoácidos de SEQ ID NO:1, en donde dicho fragmento o variante tiene actividad de albúmina, y en donde además el fragmento o variante comprende la secuencia de aminoácidos 1-387 de SEQ ID NO:1;

(e) un fragmento o variante de una proteína terapéutica: X y albúmina que comprende la secuencia de aminoácidos de SEQ ID NO:1, en donde dicho fragmento o variante tiene una actividad biológica de la proteína terapéutica: X;

(f) un proteína terapéutica: X, o fragmento o variante de la misma, y albúmina, o fragmento o variante de la misma, entre (a) y (e), en donde la proteína terapéutica:X, o fragmento o variante de la misma, está fusionada al extremo N de albúmina, o al extremo N del fragmento o variante de albúmina;

(g) una proteína terapéutica: X, o fragmento o variante de la misma, y albúmina, o fragmento o variante de la misma, entre (a) y (e), en donde la proteína Terapéutica: X, o fragmento o variante de la misma, está fusionada al extremo C de albúmina, o al extremo C del fragmento o variante de albúmina;

(h) una proteína terapéutica: X, o fragmento o variante de la misma, y albúmina, o fragmento o variante de la misma, entre (a) y (e), en donde la proteína terapéutica: X, o fragmento o variante de la misma, está fusionada al extremo N y extremo C de albúmina, o al extremo-N y el extremo C del fragmento o variante de albúmina;

(i) una proteína terapéutica: X, o fragmento o variante de la misma, y albumina, o fragmento o variante de la misma, entre (a) y (e), la cual comprende una primera proteína terapéutica: X, o fragmento o variante de la misma, y una segunda proteína terapéutica: X, o fragmento o variante de la misma, en donde dicha primera proteina terpaéutica: X, o fragmento o variante de la misma, es diferente de dicha segunda proteína terapéutica: X, o fragmento o variante de la misma;

(j) una proteína terapéutica X, o fragmento o variante de la misma, y albúmina, o fragmento o variante de la misma, entre (a) e (i), en donde la proteína terapéutica: X, o fragmento o variante de la misma, es separada de la albúmina o el fragmento o variante de albúmina por un enlazador;

(k) una proteína terapéutica: X, o fragmento o variante de la misma, y albúmina, o fragmento o variante de la misma, entre (a) y (j), en donde la proteína de fusión de albúmina tiene la siguiente fórmula:

R1-L-R2; R2-L-R1; o R1-L-R2-L-R1,

y además en donde R1 es la proteína terapéutica: X, o fragmento o variante de la misma, L es un enlazador peptídico, y R2 es albúmina que comprende la secuencia de aminoácidos de SEQ ID NO:1 o un fragmento o variante de albúmina.

2. La proteína de fusión de albúmina de la reivindicación 1, en donde la vida en almacenamiento o estabilidad en plasma de la proteína de fusión de albúmina es mayor que la vida en almacenamiento o estabilidad en plasma de la proteína terapéutica: X, o fragmento o variante de la misma, en un estado sin fusionar.

3. La proteína de fusión de albúmina de la reivindicación 1, en donde la actividad biológica in vitro de la proteína terapéutica: X, o fragmento o variante de la misma, fusionada a la albúmina, o fragmento o variante de la misma, es mayor que la actividad biológica in vitro de la proteína terapéutica: X, o fragmento o variante de la misma, en un estado sin fusionar.

4. La proteína de fusión de albúmina de la reivindicación 1, en donde la actividad biológica in vivo de la proteína terapéutica: X, o fragmento o variante de la misma, fusionada a albúmina o variante de la misma, es mayor que la actividad biológica in vivo de la proteína terapéutica: X, o o fragmento o variante de la misma, en un estado sin fusionar.

5. Una proteína de fusión de albúmina que comprende una proteína terapéutica: X, o fragmento o variante de la misma, insertada en una albúmina, o fragmento o variante de la misma, la cual comprende la secuencia de aminoácidos de SEQ ID NO: 1 o fragmento o variante de la misma.

6. Una proteína de fusión de albúmina que comprende una proteína terapéutica: X, o fragmento o variante de la misma, insertada en una albúmina, o fragmento o variante de la misma, la cual comprende una secuencia de aminoácidos seleccionada del grupo que consiste de:

(a) aminoácidos 54 a 61 de SEQ ID: NO:1;
(b) aminoácidos 76 a 89 de SEQ ID: NO:1;
(c) aminoácidos 92 a 100 de SEQ ID: NO:1;
(d) aminoácidos 170 a 176 de SEQ ID: NO:1;
(e) aminoácidos 247 a 252 de SEQ ID: NO:1;
(f) aminoácidos 266 a 277 de SEQ ID NO: 1;
(g) aminoácidos 280 a 288 de SEQ ID: NO:1;
(h) aminoácidos 362 a 368 de SEQ ID NO: 1;
(i) aminoácidos 439 a 447 de SEQ ID: NO:1;
(j) aminoácidos 462 a 475 de SEQ ID: NO:1;
(k) aminoácidos 478 a 486 de SEQ ID: NO:1; y
(l) aminoácidos 500 a 566 de SEQ ID: NO:1.

7. La proteína de fusión de albúmina de la reivindicación 5, en donde dicha proteína de fusión de albúmina comprende una porción de albúmina suficiente para prolongar la vida en almacenamiento o estabilidad en plasma de la proteína terapéutica: X, o fragmento o variante de la misma, al compararse con la vida en almacenamiento o estabilidad en plasma de la proteína terapéutica: X, o fragmento o variante de la misma, en un estado sin fusionar.

8. La proteína de fusión de albúmina de la reivindicación 6, en donde dicha proteína de fusión de albúmina comprende una porción de albúmina suficiente para prolongar la vida en almacenamiento o estabilidad en plasma de la proteína terapéutica: X, o fragmento o variante de la misma, al compararse con la vida en almacenamiento o estabilidad en plasma de la proteína terapéutica: X, o fragmento o variante de la misma, en un estado sin fusionar.

9. La proteína de fusión de albúmina de la reivindicación 5, en donde dicha proteína de fusión de albúmina comprende una porción de albúmina suficiente para prolongar la actividad biológica in vitro de la proteína terapéutica: X, o fragmento o variante de la misma, fusionada a la albúmina, al compararse con la actividad biológica in vitro de la proteína terapéutica: X, o fragmento o variante de la misma, en un estado sin fusionar.

10. La proteína de fusión de albúmina de la reivindicación 6, en donde dicha proteína de fusión de albúmina comprende una porción de albúmina suficiente para prolongar la actividad biológica in vitro de la proteína terapéutica: X, o fragmento o variante de la misma, fusionada a la albúmina, al compararse con la actividad biológica in vitro de la proteína terapéutica: X, o fragmento o variante de la misma, en un estado sin fusionar.

11. La proteína de fusión de albúmina de la reivindicación 5, en donde dicha proteína de fusión de albúmina comprende una porción de albúmina suficiente para prolongar la actividad biológica in vivo de la proteína terapéutica: X, o fragmento o variante de la misma, fusionada a la albúmina, al compararse con la actividad biológica in vivo de la proteína terapéutica: X, o fragmento o variante de la misma, en un estado sin fusionar.

12. La proteína de fusión de albúmina de la reivindicación 6, en donde dicha proteína de fusión de albúmina comprende una porción de albúmina suficiente para prolongar la actividad biológica in vivo de la proteína terapéutica: X, o fragmento o variante de la misma, fusionada a la albúmina, al compararse con la actividad biológica in vivo de la proteína terapéutica: X, o fragmento o variante de la misma, en un estado sin fusionar.

13. La proteína de fusión de albúmina de cualquiera de las reivindicaciones 1-12, la cual es no-glicosilada.

14. La proteína de fusión de albúmina de cualquiera de las reivindicaciones 1-12, la cual es expresada en levadura.

15. La proteína de fusión de albúmina de la reivindicación 14, en donde la levadura presenta glicosilación deficiente.

16. La proteína de fusión de albúmina de la reivindicación 14 en donde la levadura presenta deficiencia en glicosilación y proteasas.

17. La proteína de fusión de albúmina de cualquiera de las reivindicaciones 1-12, la cual es expresada mediante una célula mamífera.

18. La proteína de fusión de albúmina de cualquieras de las reivindicaciones 1-12, en donde la proteína de fusión de albúmina es expresada por una célula mamífera en cultivo.

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19. La proteína de fusión de albúmina de cualquiera de las reivindicaciones 1-12, en donde la proteína de fusión de albúmina además comprende una secuencia guía de secreción.

20. Una composición que comprende la proteína de fusión de albúmina de cualquiera de las reivindicaciones 1-12 y un vehículo farmacéuticamente aceptable.

21. Un kit que comprende la composición de la reivindicación 20.

22. Un método para tratar una enfermedad o trastorno en un paciente, el cual comprende el paso de administrar la proteína de fusión de albúmina de cualquiera de las reivindicaciones 1-12.

23. Un méodo de la reivindicación 22, en donde la enfermedad o trastorno comprende indicación: Y.

24. Un método para tratar un paciente con una enfermedad o trastorno que es modulado por la proteína terapéutica: X, o fragmento o variante de la misma, el cual comprende el paso de administrar una cantidad efectiva de la proteína de fusión de albúmina según cualquiera de las reivindicaciones 1-12.

25. El método de la reivindicación 24, en donde la enfermedad o trastorno es la indicación: Y.

26. Un método para extender la vida en almacenamiento o estabilidad en plasma de la proteína Terapéutica: X, o fragmento o variante de la misma, el cual comprende el paso de fusionar la proteína terapéutica:X, o fragmento o variante de la misma, con la albúmina o fragmento o variante de la misma, lo suficiente para extender la vida en almacenamiento o estabilidad en plasma de la proteína terapéutica: X, o fragmento o variante de la misma, en comparación con la vida en almacenamiento de la proteína Terapéutica: X, o fragmento o variante de la misma, en un estado sin fusionar.

27. Una molécula de ácido nucleico que comprende una secuencia polinucleótida que codifica la proteína de fusión de albúmina de cualqueira de las reivindicaciones 1-12.

28. Un vector que comprende la molécula de ácido nucleico de la reivindicación 27.

29. Una célula huésped que comprende la molécula de ácido nucleico de la reivindicación 28.

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
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REQUISITOS MINIMOS PARA ADMISION A TRAMITE
PCT

PATENTE DE INVENCION [1] MODELO DE UTILIDAD [ ]

- Indicación de que se solicita la concesión de una patente [1]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [1]
- Descripción de la invención [1]
- Dibujos de ser estos pertinentes [ ]
- Comprobante de pago de las tasas establecidas [1]

COMPLETA [ ] INCOMPLETA [ ]

DISEÑO INDUSTRIAL [ ]

- Indicación de que se solicita el registro de Diseño Industrial [ ]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [ ]
- Representación gráfica y fotográfica del Diseño Industrial o muestra del material que incorpora el diseño [ ]
- Comprobante de pago de las tasas establecidas [ ]

COMPLETA [ ] INCOMPLETA [ ]

ESQUEMA DE TRAZADO [ ]

- Indicación de que se solicita el registro de un Esquema de Trazado [ ]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [ ]
- Representación gráfica del esquema trazado [ ]
- Comprobante de pago de las tasas establecidas [ ]

COMPLETA [ ] INCOMPLETA [ ]

Firma Responsable

Fecha 07 SET. 2006

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Industria y Comercio
SUPERINTENDENCIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
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Eve: 378 FASENACIONALI
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PETITORIO - FORMULARIO ÚNICO DE SOLICITUD
PATENTE PCT
ENTRADA EN FASE NACIONAL 09/09/06

SOLICITUD DE:

[X] Patente de Invención. [ ] Patente de Modelo de Utilidad.

[X] Capítulo I. [ ] Capítulo II.

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Lugar de Constitución: ESTADOUNIDENSE
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E-mail: _____

IDENTIFICACIÓN
C.C. [ ] NIT [ ]
C.E. [ ] Otro [ ]
Cual _____
Número _____

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IDENTIFICACIÓN
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C.E. [ ] Otro [ ]
Cual _____
Número 79.376.996Bta
TP 135.799

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Nacionalidad o Domicilio: VER PAGINA ADJUNTA

PCT (86)

Solicitud Internacional No. PCT/US2005/004041 Fecha Febrero 9, 2005

Publicación Internacional No. WO 2005/077042 Fecha Agosto 25, 2005

Título (54) PROTEINAS DE FUSION DE ALBUMINA

Clasificación Internacional (51) A61K38/02

Prioridad SI [X] N [ ]

(33) País de Origen	(32) Fecha	(31) Número de Solicitud
US	Febrero 9, 2004	60/542,274
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US	Diciembre 17,2004	60/636,603

Comprobante de pago No. 06 – 68,380 **Fecha** Septiembre 6, 2006

REIVINDICACIONES

1. Una proteína de fusión de albúmina que comprende un miembro seleccionado del grupo que consta de:

(a) una proteína terapéutica: X y albúmina que comprende la secuencia de aminoácidos de SEQ ID NO:1;

(b) una proteína terapéutica: X y un fragmento o una variante de la secuencia de aminoácidos de SEQ ID NO:1, en donde dicho fragmento o variante tiene actividad de albúmina;

(c) una proteína terapéutica: X y un fragmento o una variante de la secuencia de aminoácidos de SEQ ID NO: 1, en donde dicho fragmento o variante tiene actividad de albúmina, y en donde además dicha actividad de albúmina es la capacidad para prolongar la vida en almacenamiento de la proteína terapéutica: X en comparación con la vida en almacenamiento de la proteína terapéutica: X en un estado sin fusionar;

(d) una proteína terapéutica: X y un fragmento o una variante de la secuencia de aminoácidos de SEQ ID NO:1, en donde dicho fragmento o variante tiene actividad de albúmina, y en donde además el fragmento o variante comprende la secuencia de aminoácidos 1-387 de SEQ ID NO:1;

(e) un fragmento o variante de una proteína terapéutica: X y albúmina que comprende la secuencia de aminoácidos de SEQ ID NO:1, en donde dicho fragmento o variante tiene una actividad biológica de la proteína terapéutica: X;

(f) un proteína terapéutica: X, o fragmento o variante de la misma, y albúmina, o fragmento o variante de la misma, entre (a) y (e), en donde la proteína terapéutica:X, o fragmento o variante de la misma, está fusionada al extremo N de albúmina, o al extremo N del fragmento o variante de albúmina;

(g) una proteína terapéutica: X, o fragmento o variante de la misma, y albúmina, o fragmento o variante de la misma, entre (a) y (e), en donde la proteína Terapéutica: X, o fragmento o variante de la misma, está fusionada al extremo C de albúmina, o al extremo C del fragmento o variante de albúmina;

(h) una proteína terapéutica: X, o fragmento o variante de la misma, y albúmina, o fragmento o variante de la misma, entre (a) y (e), en donde la proteína terapéutica: X, o fragmento o variante de la misma, está fusionada al extremo N y extremo C de albúmina, o al extremo-N y el extremo C del fragmento o variante de albúmina;

(i) una proteína terapéutica: X, o fragmento o variante de la misma, y albumina, o fragmento o variante de la misma, entre (a) y (e), la cual comprende una primera proteína terapéutica: X, o fragmento o variante de la misma, y una segunda proteína terapéutica: X, o fragmento o variante de la misma, en donde dicha primera proteina terpaéutica: X, o fragmento o variante de la misma, es diferente de dicha segunda proteína terapéutica: X, o fragmento o variante de la misma;

(j) una proteína terapéutica X, o fragmento o variante de la misma, y albúmina, o fragmento o variante de la misma, entre (a) e (i), en donde la proteína terapéutica: X, o fragmento o variante de la misma, es separada de la albúmina o el fragmento o variante de albúmina por un enlazador;

(k) una proteína terapéutica: X, o fragmento o variante de la misma, y albúmina, o fragmento o variante de la misma, entre (a) y (j), en donde la proteína de fusión de albúmina tiene la siguiente fórmula:

R1-L-R2; R2-L-R1; o R1-L-R2-L-R1,

y además en donde R1 es la proteína terapéutica: X, o fragmento o variante de la misma, L es un enlazador peptídico, y R2 es albúmina que comprende la secuencia de aminoácidos de SEQ ID NO:1 o un fragmento o variante de albúmina.

2. La proteína de fusión de albúmina de la reivindicación 1, en donde la vida en almacenamiento o estabilidad en plasma de la proteína de fusión de albúmina es mayor que la vida en almacenamiento o estabilidad en plasma de la proteína terapéutica: X, o fragmento o variante de la misma, en un estado sin fusionar.

3. La proteína de fusión de albúmina de la reivindicación 1, en donde la actividad biológica in vitro de la proteína terapéutica: X, o fragmento o variante de la misma, fusionada a la albúmina, o fragmento o variante de la misma, es mayor que la actividad biológica in vitro de la proteína terapéutica: X, o fragmento o variante de la misma, en un estado sin fusionar.

4. La proteína de fusión de albúmina de la reivindicación 1, en donde la actividad biológica in vivo de la proteína terapéutica: X, o fragmento o variante de la misma, fusionada a albúmina o variante de la misma, es mayor que la actividad biológica in vivo de la proteína terapéutica: X, o o fragmento o variante de la misma, en un estado sin fusionar.

5. Una proteína de fusión de albúmina que comprende una proteína terapéutica: X, o fragmento o variante de la misma, insertada en una albúmina, o fragmento o variante de la misma, la cual comprende la secuencia de aminoácidos de SEQ ID NO: 1 o fragmento o variante de la misma.

6. Una proteína de fusión de albúmina que comprende una proteína terapéutica: X, o fragmento o variante de la misma, insertada en una albúmina, o fragmento o variante de la misma, la cual comprende una secuencia de aminoácidos seleccionada del grupo que consiste de:

(a) aminoácidos 54 a 61 de SEQ ID: NO:1;
(b) aminoácidos 76 a 89 de SEQ ID: NO:1;
(c) aminoácidos 92 a 100 de SEQ ID: NO:1;
(d) aminoácidos 170 a 176 de SEQ ID: NO:1;
(e) aminoácidos 247 a 252 de SEQ ID: NO:1;
(f) aminoácidos 266 a 277 de SEQ ID NO: 1;
(g) aminoácidos 280 a 288 de SEQ ID: NO:1;
(h) aminoácidos 362 a 368 de SEQ ID NO: 1;
(i) aminoácidos 439 a 447 de SEQ ID: NO:1;
(j) aminoácidos 462 a 475 de SEQ ID: NO:1;
(k) aminoácidos 478 a 486 de SEQ ID: NO:1; y
(l) aminoácidos 500 a 566 de SEQ ID: NO:1.

7. La proteína de fusión de albúmina de la reivindicación 5, en donde dicha proteína de fusión de albúmina comprende una porción de albúmina suficiente para prolongar la vida en almacenamiento o estabilidad en plasma de la proteína terapéutica: X, o fragmento o variante de la misma, al compararse con la vida en almacenamiento o estabilidad en plasma de la proteína terapéutica: X, o fragmento o variante de la misma, en un estado sin fusionar.

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REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Bogotá,
2020

Doctor(a)
RAISBECK J. IAN

REFERENCIA	Radicación No.	6 89815
	Solicitud internacional	PCT/US2005004041
	Fecha inicio fase nacional	09/09/2006
	Trámite	11
	Evento	378
	Actuación	430
	Oficio No.	12770

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

1. La solicitud no contiene la copia del documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante. (Art. 26-k) Decisión 486.

2. La solicitud no contiene el poder debidamente otorgado, con el lleno de los requisitos exigidos por los artículos 63 y subsiguientes del Código de Procedimiento Civil. Adicionalmente, deberá presentar el documento que acredita la existencia y representación legal de la sociedad solicitante, cuando ésta fuere persona jurídica.

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Unica No.10 de 2001.

ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la división de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista de fecha 03 NOV. 2006
aalvarado