Industria y Comercio
SUPERINTENDENCIA

P

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 07-121403- -00000-0000
Fecha: 2007-11-16 17:44:01 Dep: 2020 NUECREACIONE
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACIÓN Folios: 1424

FORMULARIO UNICO DE SOLICITUD DE PATENTE PCT ENTRADA EN FASE NACIONAL 18 de 2007

1 SOLICITUD DE:

☒ Patente de Invención. ☐ Patente de Modelo de Utilidad.
☒ Capítulo I. ☐ Capítulo II.

2 SOLICITANTE (71)

Nombre: **ABLYNX NV.**
sociedad constituida y existente bajo las leyes de Bélgica.

Dirección: Technologiepark 4,
B-9052 Zwijnaarde,
Bélgica

Domicilio: B-9052 Zwijnaarde,
Bélgica

IDENTIFICACIÓN
C.C. ☐ NIT ☐
C.E. ☐ Otro ☐
Cual _____
Número _____

3 REPRESENTANTE O APODERADO

Nombre: EMILIO FERRERO
Dirección: Carrera 4ª. No. 72-35
Teléfono: 347 36 11
Fax: 211 86 50
E-mail: clientes@camyp.com.
Domicilio: Bogotá, Colombia.

IDENTIFICACIÓN
C.C. ☒ NIT ☐
C.E. ☐ Otro ☐
Cual _____
Número 438.019 T.P 31.929

4 INVENTOR (ES) (72)

1. **Els BEIRNAERT**
Marketterstraat 20A,
B-9881 Bellem,
Bélgica

5 PCT (86)

Solicitud Internacional No. PCT/EP2006/004678 Fecha: 17 de mayo de 2006
Publicación Internacional No. WO 2006/122786 A3 Fecha: 23 de noviembre de 2006

6 Título (54)

NANOBODIES™ (NANOCUERPOS) MEJORADOS CONTRA EL FACTOR ALFA DE NECROSIS DEL TUMOR

7 Clasificación Internacional (51) C07K 016/018

8 Prioridad SI☒ NO☐

(33) País de Origen	(32) Fecha	(31) Número de Solicitud
US	18 de mayo de 2005	60/682,332

9 Comprobante de pago No.: 07-91,012 **Fecha:** 7 de noviembre de 2007

10

Anexos:

- [x] Comprobante de pago de la tasa de presentación de la solicitud por $ 482.000.oo.
- [ ] Comprobante de pago por reivindicación de prioridad.
- [ ] 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 efectuado por la IPEA.
- [ ] Traducción del examen preliminar 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 12x12
- [ ] 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.
- [x] Otros: VER HOJA DE DOCUMENTOS ANEXOS

11

FIGURA CARACTERÍSTICA

12

Solicito la concesión de la patente,

NOMBRE: EMILIO FERRERO

FIRMA : _____

C.C. 438.019 de Usaquén T.P. 31.929

SCT

DOCUMENTOS ANEXOS

1. ☒ Publicación Internacional WO 2006/122786 A3

   Descripción:

   Páginas 468 en el idioma original

   Páginas 558 en español

   Reivindicaciones: Número (s) 111

   Figuras: Número (s)

2. ☒ **Forma PCT/ISA/210.** Informe de Búsqueda Internacional.

3. ☒Extracto de publicación.

NO SE REALIZARON MODIFICACIONES EN LA FASE INTERNACIONAL

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 November 2006 (23.11.2006)

PCT

(10) International Publication Number
WO 2006/122786 A3

(51) International Patent Classification:
C07K 16/18 (2006.01) *A61P 29/00* (2006.01)
C07K 16/24 (2006.01)

(21) International Application Number:
PCT/EP2006/004678

(22) International Filing Date: 17 May 2006 (17.05.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/682,332 18 May 2005 (18.05.2005) US

(71) Applicant *(for all designated States except US)*: **ABLYNX NV** [BE/BE]; Technologiepark 4, B-9052 Zwijnaarde (BE).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **BEIRNAERT, Els** [BE/BE]; Markettestraat 20A, B-9881 Bellem (BE).

(74) Agent: **TAOMINO, Joseph**; HOFFMANN EITLE, Arabellstrasse 4, 81925 Munich (DE).

(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, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report*

(88) Date of publication of the international search report:
22 March 2007

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.

(54) Title: IMPROVED NANOBODIES™ AGAINST TUMOR NECROSIS FACTOR-ALPHA

(57) **Abstract:** The present invention relates to improved Nanobodies™ against Tumor Necrosis Factor-alpha (TNF-alpha), as well as to polypeptides comprising or essentially consisting of one or more of such Nanobodies. The invention also relates to nucleic acids encoding such Nanobodies and polypeptides; to methods for preparing such Nanobodies and polypeptides; to host cells expressing or capable of expressing such Nanobodies or polypeptides; to compositions comprising such Nanobodies, polypeptides, nucleic acids or host cells; and to uses of such Nanobodies, such polypeptides, such nucleic acids, such host cells or such compositions, in particular for prophylactic, therapeutic or diagnostic purposes, such as the prophylactic, therapeutic or diagnostic purposes.

WO 2006/122786 A3

Improved Nanobodies™ against Tumor Necrosis Factor-alpha

The present invention relates to improved Nanobodies™ against Tumor Necrosis Factor-alpha (TNF-alpha), as well as to polypeptides comprising or essentially consisting of one or more of such Nanobodies. *[Note: Nanobody™, Nanobodies™ and Nanoclone™ are trademarks of Ablynx N.V.]*

The invention also relates to nucleic acids encoding such Nanobodies and polypeptides; to methods for preparing such Nanobodies and polypeptides; to host cells expressing or capable of expressing such Nanobodies or polypeptides; to compositions comprising such Nanobodies, polypeptides, nucleic acids or host cells; and to uses of such Nanobodies, such polypeptides, such nucleic acids, such host cells or such compositions, in particular for prophylactic, therapeutic or diagnostic purposes, such as the prophylactic, therapeutic or diagnostic purposes mentioned below.

Other aspects, embodiments, advantages and applications of the invention will become clear from the further description hereinbelow.

WO 04/041862 by applicant relates to Nanobodies against TNF-alpha and to the preparation and use thereof, in particular for the prevention and/or treatment of diseases and disorders associated with and/or mediated by TNF-alpha, such as inflammation, rheumatoid arthritis, Crohn's disease, ulcerative colitis, inflammatory bowel syndrome, multiple sclerosis, addison's disease, autoimmune hepatitis, autoimmune parotitis, diabetes type 1, epididymitis, glomerulonephritis, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, hemolytic anemia, systemic lupus erythematosus, male infertility, multiple sclerosis, myasthenia gravis, pemphigus, psoriasis, rheumatic fever, rheumatoid arthritis, sarcoidosis, scleroderma, Sjogren's syndrome, spondyloarthropathies, thyroiditis, and vasculitis.

The anti-TNF Nanobodies according to WO 04/041862 may be humanized and may be monovalent or multivalent, the latter of which leads to increased affinity for TNF. The anti-TNF Nanobodies™ according to WO 04/041862 may also be multispecific, and may in particular be in the form of a multispecific construct comprising two or more Nanobodies against TNF and a further Nanobody directed against a serum protein such as human serum albumin, which leads to an increased half-life in vivo.

WO 04/041862 also relates to methods for the preparation of the anti-TNF Nanobodies, to nucleic acids or constructs encoding the anti-TNF Nanobodies, as well as

to pharmaceutical compositions comprising the anti-TNF Nanobodies, which may be suitable for intravenous, subcutaneous, oral, sublingual, topical, nasal, vaginal or rectal administration, or for administration by inhalation. The anti-TNF Nanobodies according to WO 04/041862 may also be used for diagnostic purposes, optionally in the form of a kit-of-parts.

EP 0 486 526 describes TNF-alpha binding ligands against a specific epitope of TNF. Among the binding ligands, single domain antibodies ("dAbs") are mentioned.

Reiter et al., *J. Mol. Biol.* (1999), 290, 685-698 describe single domain antibodies against TNF-alpha obtained from a randomized phage display library that was generated starting from a VH domain scaffold from a mouse hybridoma.

WO 00/29004 describes murine single domain antibodies ("microbodies") against TNF-alpha.

WO 04/003019 *inter alia* describes ligands comprising a first binding domain against TNF-alpha and a second binding domain against a serum protein such as serum albumin.

It is a general object of the present invention to provide Nanobodies against TNF-alpha, in particular against human TNF-alpha.

In particular, it is an object of the present invention to provide Nanobodies against TNF-alpha, in particular against human TNF-alpha, and to provide proteins or polypeptides comprising the same, that are suitable for therapeutic and/or diagnostic use, and in particular for the prevention, treatment and/or diagnosis of one or more diseases and disorders associated with and/or mediated by TNF-alpha such as those mentioned above, and/or that can be used in the preparation of a pharmaceutical composition for the prevention and/or treatment of one or more diseases associated with and/or mediated by TNF-alpha, such as those mentioned above.

More in particular, it is an object of the invention to provide Nanobodies against TNF-alpha, and to provide proteins and polypeptides comprising the same, that are either an alternative to the Nanobodies and polypeptides against TNF-alpha described in WO 04/041862 and/or that have one or more improved properties or characteristics, compared to the Nanobodies and polypeptides against TNF-alpha described in WO 04/041862.

More in particular, it is an object of the invention to provide Nanobodies against TNF-alpha, and to provide proteins or polypeptides comprising the same, that are

improved compared to the Nanobodies and polypeptides against TNF-alpha described in WO 04/041862 with respect to one or more of the following properties or characteristics:

- increased affinity for TNF-alpha, either in a monovalent format, in a multivalent format (for example in a bivalent format) and/or in a multispecific format (for example one of the multispecific formats described in WO 04/041862 or hereinbelow);
- better suitability for formatting in a multivalent format (for example in a bivalent format);
- better suitability for formatting in a multispecific format (for example one of the multispecific formats described in WO 04/041862 or hereinbelow);
- improved suitability or susceptibility for "humanizing" substitutions (as defined herein); and/or
- less immunogenicity, either in a monovalent format, in a multivalent format (for example in a bivalent format) and/or in a multispecific format (for example one of the multispecific formats described in WO 04/041862 or hereinbelow) in a monovalent format;
- increased stability, either in a monovalent format, in a multivalent format (for example in a bivalent format) and/or in a multispecific format (for example one of the multispecific formats described in WO 04/041862 or hereinbelow) in a monovalent format;
- increased specificity towards TNF-alpha, either in a monovalent format, in a multivalent format (for example in a bivalent format) and/or in a multispecific format (for example one of the multispecific formats described in WO 04/041862 or hereinbelow) in a monovalent format;
- decreased or where desired increased cross-reactivity with TNF-alpha from different species;

and/or

- one or more other improved properties desirable for pharmaceutical use (including prophylactic use and/or therapeutic use) and/or for diagnostic use (including but not limited to use for imaging purposes), either in a monovalent format, in a multivalent format (for example in a bivalent format) and/or in a multispecific format (for example one of the multispecific formats described in WO 04/041862 or hereinbelow).

These objects are achieved by the Nanobodies, proteins and polypeptides described herein. These Nanobodies are also referred to herein as "*Nanobodies of the invention*"; and these proteins and polypeptides are also collectively referred to herein "*polypeptides of the invention*".

Since the Nanobodies and polypeptides described herein are mainly intended for therapeutic and/or diagnostic use, they are directed against (as defined herein) human TNF-alpha. It is however not excluded (but also not required) that Nanobodies and polypeptides described herein show cross-reactivity with TNF-alpha from one or more other species of warm-blooded animals, for example with TNF-alpha from one or more other species of primates and/or with TNF-alpha from one or more species of animals that are often used in animal models for diseases (for example mouse, rat, rabbit, pig or dog), and in particular in animal models for diseases and disorders associated with TNF-alpha (such as the species and animal models mentioned herein). In this respect, it will be clear to the skilled person that such cross-reactivity, when present, may have advantages from a drug development point of view, since it allows the Nanobodies and polypeptides against human TNF-alpha to be tested in such disease models.

The present invention is in its broadest sense also not particularly limited to or defined by a specific antigenic determinant, epitope, part, domain, subunit or confirmation (where applicable) of TNF-alpha against which the Nanobodies and polypeptides of the invention are directed.

However, in a preferred embodiment, the Nanobodies, proteins or polypeptides described herein are directed against and/or can bind to an epitope of TNF-alpha that lies in and/or forms part of the TNF receptor binding site(s) (e.g. the binding sites for the TNF-RI, THF-RII, also known as p55 or p75). As is well known in the art, a TNF trimer comprises three receptor binding sites, which are essentially equivalent and which are formed by/at the interface of two TNF monomers within the TNF trimer. For example, the Nanobodies, proteins or polypeptides described herein are preferably directed against and/or can bind to an epitope of TNF-alpha that comprises the following amino acid residues of TNF-alpha: Gln at position 88, Lys at position 90, and/or Glu at position 146).

In particular, the Nanobodies, proteins or polypeptides described herein are directed against and/or can bind to an epitope of the TNF-alpha trimer, which lies in and/or forms part of the TNF receptor binding site(s). For example, the Nanobodies, proteins or polypeptides described herein may be directed against and/or can bind to an

epitope of the TNF-alpha trimer that comprises the following amino acid residues: Gln at position 88 and Lys at position 90 on a first TNF monomer (referred to herein as "monomer A"), and Glu at position 146 on a second TNF monomer (referred to herein as "monomer B") (in which Monomer A and Monomer B together, in the TNF trimer, form the TNF receptor binding site(s)).

More particularly, the Nanobodies, proteins or polypeptides described herein may be directed against and/or can bind to an epitope of the TNF-alpha trimer that comprises the aforementioned amino acids (Gln at position 88 in monomer A; Lys at position 90 in monomer A and Glu at position 146 in monomer B), and in addition at least one, preferably two or more, more preferably 5 or more, and preferably all or essentially all, of the following amino acid residues of TNF-alpha monomer A: Gly at position 24, Gln at position 25, Thr at position 72, His at position 73, Val at position 74, Leu at position 75, Thr at position 77, Thr at position 79, Ile at position 83, Thr at position 89, Val at position 91. Asn at position 92, Ile at position 97, Arg at position 131, Glu at position 135, Ile at position 136, Asn at position 137, Arg at position 138, Pro at position 139, Asp at position 140 and the following residues in monomer B: Pro at position 20, Arg at position 32, Lys at position 65, Lys at position 112, Tyr at position 115, Ala at position 145, Ser at position 147.

Alternatively, the Nanobodies, proteins or polypeptides described herein may be directed against and/or can bind to an epitope of TNF-alpha that comprises the aforementioned amino acids (Gln at position 88 in monomer A; Lys at position 90 in monomer A and Glu at position 146 in monomer B), and in addition at least one, preferably two or more, more preferably 5 or more and preferably all or essentially all, of the following amino acid residues of TNF-alpha monomer A: Leu at position 75, Thr at position 77, Thr at position 79, Ile at position 80, Ser at position 81, Tyr at position 87, Thr at position 89, Val at position 91, Asn at position 92, Ser at position 95, Ile at position 97, Glu at position 135, Ile at position 136, Asn at position 137 and the following residues in monomer B: Ala at position 33, Ala at position 145, Ser at position 147.

Such epitope can be delineated from structural analysis of the nanobody crystallized in complex with the TNF molecule, or from other approaches such as epitope mapping via pepscan analysis.

By comparison, from crystallographic data (not shown), it can be seen that the Nanobody 3E from WO 04/041862 binds to a different epitope (i.e. an epitope comprising

Tyr at position 141, Asp at position 140, Gln at position 67, Gly at position 24 and Glu at position 23) than the preferred epitope of the invention.

Thus, in another aspect, the present invention relates to an immunoglobulin variable domain (or a suitable fragment thereof) that can bind to an epitope of TNF-alpha that lies in and/or forms part of the TNF receptor binding site, and preferably to an epitope that comprises at least one, preferably two or more, and preferably all, of the following amino acid residues of TNF-alpha: Gln at position 88; Lys at position 90 and Glu at position 146. Such an immunoglobulin variable domain is preferably a heavy chain variable domain or a light chain variable domain, and in particular a heavy chain variable domain, which may be any mammalian heavy chain variable domain, including but not limited to human heavy chain variable domains, mouse heavy chain variable domains and Camelid heavy chain variable domains (such as the heavy chain variable domains from Camelid 4-chain immunoglobulins or the heavy chain variable domains (VHH domains) from so-called heavy chain antibodies). The immunoglobulin variable domain is preferably a domain antibody or single domain antibody or suitable for use as a (single) domain antibody. Most preferably, the immunoglobulin variable domain is a Nanobody (as defined herein), and some preferred, but non-limiting examples of Nanobodies that are suitable for use in this aspect of the invention are PMP1C2 (TNF1, SEQ ID NO:52) and PMP5F10 (TNF3, SEQ ID NO: 60), as well as humanized and other variants thereof (as further described herein).

The aforementioned immunoglobulin variable domain may also be humanized (as for example, and without limitation) described herein with respect to Nanobodies. The invention also relates to proteins and polypeptides that comprise or essentially consist of such immunoglobulin variable domains, which may for example be as defined herein. Alternatively, such variable domains may form part of ScFv constructs, dual-specific constructs, chimeric antibody or antibody structures and other immunoglobulin constructs, as for example reviewed by Hoogenboom (Nature Biotechnology (1997), 15:125-126). Preferably, however, the immunoglobulin variable domains directed against the above epitope are Nanobodies, in which case the proteins and polypeptides comprising such Nanobodies may be as further described herein.

Thus, some preferred aspects of the invention relate to:

I) A Nanobody which is directed against the same epitope on the trimer of TNF-alpha as Nanobody TNF1 (SEQ ID NO: 52).

II) A Nanobody which is directed against the same epitope on the trimer of TNF-alpha as Nanobody TNF3 (SEQ ID NO: 60).

III) A Nanobody which is directed against an epitope of the trimer of TNF-alpha that at least comprises the following amino acid residues: Gln at position 88 in monomer A; Lys at position 90 in monomer A and Glu at position 146 in monomer B.

IV) A Nanobody which is directed against an epitope of the trimer of TNF-alpha that comprises the following amino acid residues: Gln at position 88 in monomer A; Lys at position 90 in monomer A and Glu at position 146 in monomer B; and that further comprises at least comprises at least one, preferably two or more, more preferably 5 or more, and preferably all or essentially all, of the following amino acid residues of TNF-alpha monomer A: Gly at position 24, Gln at position 25, Thr at position 72, His at position 73, Val at position 74, Leu at position 75, Thr at position 77, Thr at position 79, Ile at position 83, Thr at position 89, Val at position 91. Asn at position 92, Ile at position 97, Arg at position 131, Glu at position 135, Ile at position 136, Asn at position 137, Arg at position 138, Pro at position 139, Asp at position 140 and the following residues in monomer B: Pro at position 20, Arg at position 32, Lys at position 65, Lys at position 112, Tyr at position 115, Ala at position 145, Ser at position 147.

V) A Nanobody which is directed against an epitope of the trimer of TNF-alpha that comprises the following amino acid residues: Gln at position 88 in monomer A; Lys at position 90 in monomer A and Glu at position 146 in monomer B; and that further comprises at least one, preferably two or more, more preferably 5 or more, and preferably all or essentially all, of the following amino acid residues of TNF-alpha monomer A Leu at position 75, Thr at position 77, Thr at position 79, Ile at position 80, Ser at position 81, Tyr at position 87, Thr at position 89, Val at position 91, Asn at position 92, Ser at position 95, Ile at position 97, Glu at position 135, Ile at position 136, Asn at position 137 and the following residues in monomer B: Ala at position 33, Ala at position 145, Ser at position 147.

- A Nanobody in accordance with any of of I) to V) above, which has a K_{off} rate for TNF of better than 2.10^{-3} (1/s), preferably better than 1.10^{-3}.
- A Nanobody in accordance with any one of I) to V) above , which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than the EC50 value of Nanobody VHH 3E (SEQ ID

NO:4) of WO 04/041862 in the same assay; or a humanized variant of such a Nanobody.

- A Nanobody in accordance with any one of I) to V) above , which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 12nM; or a humanized variant of such a Nanobody..
- A Nanobody in accordance with any one of I) to V) above , which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 5nM; or a humanized variant of such a Nanobody..
- A Nanobody in accordance with any one of I) to V) above , which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 3nM; or a humanized variant of such a Nanobody..
- A Nanobody in accordance with any one of I) to V) above , which is a humanized Nanobody.

and some preferred aspects of this embodiment relate to:

- A Nanobody in accordance with any one of I) to V) above , which is a GLEW-class Nanobody.
- A Nanobody in accordance with any one of I) to V) above , which is a humanized GLEW-class Nanobody.
- A Nanobody in accordance with any one of I) to V) above , which contains an arginine residue (R) at position 103.
- A Nanobody in accordance with any one of I) to V) above ,which contains an arginine residue (R) at position 103, and which is humanized
- A Nanobody in accordance with any one of I) to V) above , which is a GLEW-class Nanobody, and which contains an arginine residue (R) at position 103, and which is humanized.
- A Nanobody in accordance with any one of I) to V) above , which contains a leucine residue (L) at position 108.
- A Nanobody in accordance with any one of I) to V) above , which has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the amino acid sequences of SEQ ID NO's 52 (TNF1), 76 (TNF13), 77 (TNF14), 95 (TNF29) or 96 (TNF30)
- A Nanobody in accordance with any one of I) to V) above , in which

13

a) CDR1 comprises:

- the amino acid sequence DYWMY; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence DYWMY; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence DYWMY;

and

b) CDR2 comprises:

- the amino acid sequence EINTNGLITKYPDSVKG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence EINTNGLITKYPDSVKG; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence EINTNGLITKYPDSVKG;

and

c) CDR3 comprises:

- the amino acid sequence SPSGFN; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence SPSGFN; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence SPSGFN.

- A Nanobody in accordance with any one of I) to V) above , in which CDR1 comprises the amino acid sequence DYWMY.
- A Nanobody in accordance with any one of I) to V) above , in which CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG.
- A Nanobody in accordance with any one of I) to V) above , in which CDR3 comprises the amino acid sequence SPSGFN
- A Nanobody in accordance with any one of I) to V) above , in which:
  - CDR1 comprises the amino acid sequence DYWMY; and CDR3 comprises the amino acid sequence SPSGFN; or

- CDR1 comprises the amino acid sequence DYWMY; and CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG; or
- CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG; and CDR3 comprises the amino acid sequence SPSGFN

- A Nanobody in accordance with any one of I) to V) above , in which CDR1 comprises the amino acid sequence DYWMY; and CDR3 comprises the amino acid sequence SPSGFN.
- A Nanobody in accordance with any one of I) to V) above , in which CDR1 comprises the amino acid sequence DYWMY; CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG and CDR3 comprises the amino acid sequence SPSGFN.
- A Nanobody in accordance with any one of I) to V) above, in which

a) CDR1 is:

- the amino acid sequence DYWMY; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence DYWMY; or
- an amino acid sequences that has 2 or only 1 amino acid difference with the amino acid sequence DYWMY;

and in which:

b) CDR2 is:

- the amino acid sequence EINTNGLITKYPDSVKG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence EINTNGLITKYPDSVKG; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence EINTNGLITKYPDSVKG;

and in which

c) CDR3 is:

- the amino acid sequence SPSGFN; or

- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence SPSGFN; or
- an amino acid sequences that has 2 or only 1 amino acid difference with the amino acid sequence SPSGFN.

- A Nanobody in accordance with any one of I) to V) above , in which CDR1 is the amino acid sequence DYWMY.
- A Nanobody in accordance with any one of I) to V) above , in which CDR2 is the amino acid sequence EINTNGLITKYPDSVKG.
- A Nanobody in accordance with any one of I) to V) above , in which CDR3 is the amino acid sequence SPSGFN
- A Nanobody in accordance with any one of I) to V) above , in which:
  - CDR1 is the amino acid sequence DYWMY; and CDR3 is the amino acid sequence SPSGFN; or
  - CDR1 is the amino acid sequence DYWMY; and CDR2 is the amino acid sequence EINTNGLITKYPDSVKG; or
  - CDR2 is the amino acid sequence EINTNGLITKYPDSVKG; and CDR3 is the amino acid sequence SPSGFN
- A Nanobody in accordance with any one of I) to V) above , in which CDR1 is the amino acid sequence DYWMY; and CDR3 is the amino acid sequence SPSGFN.
- A Nanobody in accordance with any one of I) to V) above , in which CDR1 is the amino acid sequence DYWMY; CDR2 is the amino acid sequence EINTNGLITKYPDSVKG and CDR3 is the amino acid sequence SPSGFN.
- A Nanobody in accordance with any one of I) to V) above , in which
  - any amino acid substitution is preferably a conservative amino acid substitution; and/or
  - said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s).

and some other preferred aspects of this embodiment relate to:

- A Nanobody in accordance with any one of I) to V) above , which is a KERE-class Nanobody

- A Nanobody in accordance with any one of I) to V) above , which is a humanized KERE-class Nanobody
- A Nanobody in accordance with any one of I) to V) above , which has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the amino acid sequences of SEQ ID NO's 50 (TNF3), 83 (TNF20), 85 (TNF21), 85 (TNF22), 96 (TNF23) or 98 (TNF33).
- A Nanobody in accordance with any one of I) to V) above , in which

a) CDR1 is:

- the amino acid sequence NYYMG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence NYYMG; or
- an amino acid sequences that has 2 or only 1 amino acid difference with the amino acid sequence NYYMG;

and

b) CDR2 is:

- the amino acid sequence NISWRGYNIYYKDSVKG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence NISWRGYNIYYKDSVKG; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence NISWRGYNIYYKDSVKG;

and

c) CDR3 is:

- the amino acid sequence SILPLSDDPGWNTY; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence SILPLSDDPGWNTY; or
- an amino acid sequences that has 2 or only 1 amino acid difference with the amino acid sequence SILPLSDDPGWNTY.

- A Nanobody in accordance with any one of I) to V) above , in which CDR1 is the amino acid sequence NYYMG.
- A Nanobody in accordance with any one of I) to V) above , in which CDR2 is the amino acid sequence NISWRGYNIYYKDSVKG.
- A Nanobody in accordance with any one of I) to V) above , in which CDR3 is the amino acid sequence SILPLSDDPGWNTY.
- A Nanobody in accordance with any one of I) to V) above , in which:
  - CDR1 is the amino acid sequence NYYMG; and CDR3 is the amino acid sequence SILPLSDDPGWNTY; or
  - CDR1 is the amino acid sequence NYYMG; and CDR2 is the amino acid sequence NISWRGYNIYYKDSVKG; or
  - CDR2 is the amino acid sequence NISWRGYNIYYKDSVKG; and CDR3 is the amino acid sequence SILPLSDDPGWNTY.
- A Nanobody in accordance with any one of I) to V) above , in which CDR1 is the amino acid sequence NYYMG; CDR2 is the amino acid sequence SILPLSDDPGWNTY and CDR3 is the amino acid sequence ILPLSDDPGWNTY.
- A Nanobody in accordance with any one of I) to V) above , in which
  - any amino acid substitution is preferably a conservative amino acid substitution; and/or
  - said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s).

and with yet some other particularly preferred aspects being:

VI) A protein or polypeptide, which comprises a or essentially consists a Nanobody in accordance with any one of I) to V) above .

VII) A protein or polypeptide, which comprises or essentially consists of at least one Nanobody in accordance with any one of I) to V) above .

VIII) A protein or polypeptide, which comprises two Nanobodies in accordance with any one of I) to V) above .

IX) A protein or polypeptide, which comprises two Nanobodies in accordance with any one of I) to V) above , and which is such that said protein or polypeptide, upon binding to a TNF trimer, is capable inhibiting or reducing the TNF receptor

crosslinking that is mediated by said TNF trimer and/or the signal transduction that is mediated by such receptor crosslinking..

X) A protein or polypeptide, which comprises two Nanobodies in accordance with any one of I) to V) above , and which is capable of intramolecular binding to at least two TNF receptor binding sites on a TNF trimer.

XI) A protein or polypeptide, which comprises two Nanobodies in accordance with any one of I) to V) above , linked via a suitable linker.

XII) A protein or polypeptide, which comprises two Nanobodies in accordance with any one of I) to V) above , linked via a suitable linker, and which is pegylated.

XIII) A protein or polypeptide which comprises two Nanobodies in accordance with any one of I) to V) above , and which further comprises at least one Nanobody directed against human serum albumin.

XIV) A protein or polypeptide which comprises two Nanobodies in accordance with any one of I) to V) above , and which further comprises at least one Nanobody directed against human serum albumin, and which is such that said protein or polypeptide, upon binding to a TNF trimer, is capable inhibiting or reducing the TNF receptor crosslinking that is mediated by said TNF trimer and/or the signal transduction that is mediated by such receptor crosslinking..

XV) A protein or polypeptide which comprises two Nanobodies in accordance with any one of I) to V) above , and which further comprises at least one Nanobody directed against human serum albumin and which is capable of intramolecular binding to at least two TNF receptor binding sites on a TNF trimer.

XVI) A protein or polypeptide which comprises two Nanobodies in accordance with any one of I) to V) above , and which further comprises one Nanobody directed against human serum albumin, in which each of the two Nanobodies in accordance with any one of I) to V) above is linked, optionally via a suitable linker, to the one Nanobody directed against human serum albumin.

XVII) A protein or polypeptide which comprises two Nanobodies in accordance with any one of I) to V) above , and which further comprises one Nanobody directed against human serum albumin, in which each of the two Nanobodies in accordance with any one of I) to V) above is linked, optionally via a suitable linker, to the one Nanobody directed against human serum albumin, and which is such that said protein or polypeptide, upon binding to a TNF trimer, is capable inhibiting or reducing the

TNF receptor crosslinking that is mediated by said TNF trimer and/or the signal transduction that is mediated by such receptor crosslinking..

XVIII) A protein or polypeptide which comprises two Nanobodies in accordance with any one of I) to V) above, and which further comprises one Nanobody directed against human serum albumin, in which each of the two Nanobodies in accordance with any one of I) to V) above is linked, optionally via a suitable linker, to the one Nanobody directed against human serum albumin, and which is capable of intramolecular binding to at least two TNF receptor binding sites on a TNF trimer.

- A protein or polypeptide in accordance with any one of VI) to XVIII) above , in which the at least one Nanobody directed against human serum albumin is a humanized Nanobody.
- A protein or polypeptide in accordance with any one of VI) to XVIII) above , in which the at least one Nanobody directed against human serum albumin is a humanized variant of the Nanobody ALB 1 (SEQ ID NO: 63).
- A protein or polypeptide in accordance with any one of VI) to XVIII) above , in which the at least one Nanobody directed against human serum albumin is a chosen from the group consisting of ALB 3 (SEQ ID NO: 87), ALB 4 (SEQ ID NO: 88), ALB 5 (SEQ ID NO: 89), ALB 6 (SEQ ID NO: 100), ALB 7 (SEQ ID NO: 101), ALB 8 (SEQ ID NO: 102) ALB 9 (SEQ ID NO: 103) and ALB 10 (SEQ ID NO: 104).
- A protein or polypeptide in accordance with any one of VI) to XVIII) above , in which the at least one Nanobody directed against human serum albumin is ALB 8.
- A protein or polypeptide in accordance with any one of VI) to XVIII) above , which comprises or essentially consists of two humanized Nanobodies in accordance with any one of I) to V) above , and one humanized variant of the Nanobody ALB 1 (SEQ ID NO: 63).

It should be noted that when a Nanobody is mentioned above as being "*in accordance with any one of I) to V) above*", it is at least according to one of I) to V), may be according to two or more of I) to V), and may also include any one or more of the other aspects that are indicated as being "in accordance with any one of I) to V) above. Similarly, when a protein or polypeptide is mentioned above as being "*in accordance with any one of VI) to XVIII) above*", it is at least according to one of VI) to XVIII), may be according to two or more of VI) to XVIII), and may also include any one or more of the

other aspects that are indicated as being "in accordance with any one of VI) to XVIII) above.

It is also within the scope of the invention that, where applicable, a Nanobody or polypeptide of the invention can bind to two or more antigenic determinants, epitopes, parts, domains, subunits or confirmations of TNF-alpha. In such a case, the antigenic determinants, epitopes, parts, domains or subunits of TNF-alpha to which the Nanobodies and/or polypeptides of the invention bind may be the essentially same (for example, if TNF-alpha contains repeated structural motifs or is present as a multimer) or may be different (and in the latter case, the Nanobodies and polypeptides of the invention may bind to such different antigenic determinants, epitopes, parts, domains, subunits of TNF-alpha with an affinity and/or specificity which may be the same or different). Also, for example, when TNF-alpha exists in an activated conformation and in an inactive conformation, the Nanobodies and polypeptides of the invention may bind to either one of these conformation, or may bind to both these conformations (i.e. with an affinity and/or specificity which may be the same or different). Also, for example, the Nanobodies and polypeptides of the invention may bind to a conformation of TNF-alpha in which it is bound to a pertinent ligand, may bind to a conformation of TNF-alpha in which it not bound to a pertinent ligand, or may bind to both such conformations (again with an affinity and/or specificity which may be the same or different).

It is also expected that the Nanobodies and polypeptides of the invention will generally bind to all naturally occurring or synthetic analogs, variants, mutants, alleles, parts and fragments of TNF-alpha, or at least to those analogs, variants, mutants, alleles, parts and fragments of TNF-alpha that contain one or more antigenic determinants or epitopes that are essentially the same as the antigenic determinant(s) or epitope(s) to which the Nanobodies and polypeptides of the invention bind in TNF-alpha (e.g. in wild-type TNF-alpha). Again, in such a case, the Nanobodies and polypeptides of the invention may bind to such analogs, variants, mutants, alleles, parts and fragments with an affinity and/or specificity that are the same as, or that different from (i.e. higher than or lower than), the affinity and specificity with which the Nanobodies of the invention bind to (wild-type) TNF-alpha. It is also included within the scope of the invention that the Nanobodies and polypeptides of the invention bind to some analogs, variants, mutants, alleles, parts and fragments of TNF-alpha, but not to others.

Generally, the Nanobodies and polypeptides of the invention will at least bind to those forms (including monomeric, multimeric and associated forms) that are the most relevant from a biological and/or therapeutic point of view, as will be clear to the skilled person.

Also, as TNF-alpha exists in a monomeric form and in multimeric forms, and in particular in trimeric form, it is within the scope of the invention that the Nanobodies and polypeptides of the invention only bind to TNF-alpha in monomeric form, or that the Nanobodies and polypeptides of the invention in addition also bind to one or more of such multimeric forms, such as the trimeric form of TNF; or may only bind to such a multimeric (e.g. trimeric) form. Thus, generally, when in this description reference is made to a Nanobody, protein or polypeptide that is directed to TNF-alpha, it should be understood that this also comprises Nanobodies directed against TNF-alpha in its trimeric form (including but not limited to Nanobodies against the receptor binding sites (e.g. the binding sites for the TNF-RI, THF-RII, also known as p55 or p75) of such a trimer). In all these cases, the Nanobodies and polypeptides of the invention may bind to such multimers or associated protein complexes with an affinity and/or specificity that may be the same as or different from (i.e. higher than or lower than) the affinity and/or specificity with which the Nanobodies and polypeptides of the invention bind to TNF-alpha in its monomeric and non-associated state.

Also, generally, polypeptides of the invention that contain two or more Nanobodies directed against TNF-alpha may bind with higher avidity than the corresponding monomeric Nanobody or Nanobodies.

For example, and without limitation, a multivalent (as defined herein) protein or polypeptide that contains two or more Nanobodies that are directed against different epitopes of TNF-alpha multivalent (as defined herein) protein or polypeptide that contains two or more Nanobodies that are directed against different epitopes of TNF-alpha may bind to TNF-alpha with higher avidity than the corresponding monomers.

More importantly, a multivalent (as defined herein) protein or polypeptide that contains two or more Nanobodies that are directed against TNF-alpha may (and usually will) bind with higher avidity to a multimer of TNF-alpha than to a monomer of TNF-alpha, and will usually also bind with higher avidity than the corresponding monomeric Nanobodies. In such a multivalent protein or polypeptide, the two or more Nanobodies may for example be directed against the same epitopes, substantially equivalent epitopes,

or different epitopes. In one embodiment of such a multivalent protein or polypeptide, the two or more Nanobodies may be the same (and therefore be directed against the same epitope).

The latter is of particular importance, as it is known that the primary mode of signal transduction by TNF involves crosslinking by TNF receptors by a trimer of TNF molecules, which contains three receptor binding sites (see for example Peppel et al., *J. Exp. Med.*, 174 (1991), 1483-1489; Engelmann et al., *J. Biol. Chem.*, 265 (1990), 14497; Smith and Baglioni, *J. Biol. Chem.*, 264 (1989), 14646). For example, as described by Peppel et al., an engineered monovalent extracellular domain of the TNF receptor - which was only capable of blocking a single receptor binding site on aTNF trimer - was unable to prevent crosslinking of the TNF receptors by the remaining two receptor binding sites; whereas an engineered protein that comprises two such extracellular domains – thus being capable of blocking two receptor binding sites – provided a striking efficacy compared to the monovalent extracellular domain.

In the present invention, it has been found that monovalent Nanobodies are capable of binding to TNF alpha in such a way that the activity of TNF is reduced, both in in vitro models, in cellular models and in ex vivo models (see the Experimental Section below). Although the invention is not limited to any specific mechanism, explanation or hypothesis, it is assumed that because of their small size and high affinity for TNF-alpha, two or three monovalent Nanobodies of the invention are capable of simultaneously occupying two or three different receptor binding sites on the TNF trimer, thus preventing the trimer to initiate receptor crosslinking and thereby to initiate signal transduction (however, other mechanisms of action are not excluded: for example, depending on the epitope against which it is directed, a Nanobody of the invention may also inhibit the association of TNF into the trimeric state).

It should also be noted that, in addition or as an alternative to binding to two or more receptor binding sites on a single TNF-trimer, the proteins or polypeptides of the present invention that comprises or essentially consists of two or more immunoglobulin variable domains (or suitable fragments thereof) that are directed against epitopes of TNF-alpha may bind (e.g. intermolecularly) epitopes on two separate TNF-alpha molecules (e.g. two separate trimers).

However, according to one particularly preferred embodiment, the invention relates to a protein or polypeptide that comprises or essentially consists of two or more

immunoglobulin variable domains (or suitable fragments thereof) that are each directed against epitopes on TNF-alpha (and in particular of the TNF-alpha trimer) that lie in and/or form part of the receptor binding site(s) of the TNF trimer, such that said polypeptide, upon binding to a TNF trimer, is capable inhibiting or reducing the TNF receptor crosslinking that is mediated by said TNF trimer and/or the signal transduction that is mediated by such receptor crosslinking.

In particular, according to this preferred embodiment, the invention relates to a protein polypeptide that comprises or essentially consist of two or more immunoglobulin variable domains (or suitable fragments thereof) that are each directed against epitopes on TNF-alpha (and in particular of the TNF-alpha trimer) that lie in and/or form part of the receptor binding site(s) of the TNF-trimer, wherein said immunoglobulin variable domains are linked to each other in such a way that the protein or polypeptide is capable of simultaneously binding to two or more receptor binding sites on a single TNF trimer (in other words, is capable of intramolecular binding to at least two TNF receptor binding sites on a TNF trimer). In this embodiment, the two or more immunoglobulin variable domains are preferably as defined above and are most preferably Nanobodies (so that the protein or polypeptide is a multivalent Nanobody construct, as further described herein). Also, in this embodiment, the two or more immunoglobulin variable domains may be the same or different; and may directed against different epitopes within the TNF receptor binding site(s), but are preferably directed against the same epitope.

In one preferred aspect of this embodiment, the two or more immunoglobulin variable domains are directed against epitopes of the TNF-alpha trimer, which epitopes lie in and/or form part of the TNF receptor binding site(s). For example, the two or more immunoglobulin variable domains are preferably directed against and/or can bind to an epitope of the TNF-alpha trimer that comprises the following amino acid residues: Gln at position 88 and Lys at position 90 on a first TNF monomer (referred to herein as "monomer A"), and Glu at position 146 on a second TNF monomer (referred to herein as "monomer B") (in which Monomer A and Monomer B together, in the TNF trimer, form the TNF receptor binding site(s)).

As further described below in more details with respect to Nanobodies, in such a protein or polypeptide, the at least two immunoglobulin variable domains are preferably linked in such a way that the distance between the N-terminus and the C-terminus of the two immunoglobulin variable domains present in such a protein or polypeptide is

preferably at least 50 Angstroms, and more preferably in the region of 55-200 Angstroms, and more preferably in the region of Angstroms, and in particular in the region of 65-150 Angstroms.

In a particularly preferred aspect of this embodiment, these two or more immunoglobulin sequences are Nanobodies, and are preferably chosen from the Nanobodies described herein. Some particularly preferred Nanobodies for use in this embodiment of the invention are PMP1C2 (TNF1, SEQ ID NO:52) and/or PMP5F10 (TNF3, SEQ ID NO: 60), as well as humanized and other variants thereof (as described herein); with PMP1C2 (TNF1, SEQ ID NO:52) and its humanized variants being particularly preferred.

Accordingly, the present embodiment will now be described in more detail with reference to Nanobodies. However, it will be clear to the skilled person that the teaching herein may be applied analogously to immunoglobulin variable domains.

In this embodiment of the invention, the two or more immunoglobulin sequences will usually be linked via one or more suitable linkers, which linkers are such that each immunoglobulin sequence can bind to a different receptor binding site on the same TNF trimer. Suitable linkers will inter alia depend on (the distance between) the epitopes on the TNF trimer to which the immunoglobulin sequences bind, and will be clear to the skilled person based on the disclosure herein, optionally after some limited degree of routine experimentation. For example, when the two or more immunoglobulin sequences are (single) domain antibodies or Nanobodies, suitable linkers may be chosen from the linkers described herein, but with a linker length that is such that the two or more (single) domain antibodies or Nanobodies can each bind to a different receptor binding site on the same TNF trimer.

Also, when the two or more immunoglobulin sequences that bind to the receptor binding sites of TNF-alpha are (single) domain antibodies or Nanobodies, they may also be linked to each other via a third (single) domain antibody or Nanobody (in which the two or more immunoglobulin sequences may be linked directly to the third (single) domain antibody/Nanobody or via suitable linkers). Such a third (single) domain antibody or Nanobody may for example be a (single) domain antibody or Nanobody that provides for an increased half-life, as further described herein. For example, the latter (single) domain antibody or Nanobody may be a (single) domain antibody or Nanobody that is

capable of binding to a (human) serum protein such as (human) serum albumin, as further described herein.

Alternatively, the two or more immunoglobulin sequences that bind to the receptor binding site(s) of TNF-alpha may be linked in series (either directly or via a suitable linker) and the third (single) domain antibody or Nanobody (which may provide for increased half-life, as decribed above) may be connected directly or via a linker to one of these two or more aforementioned immunoglobulin sequences. Some non-limiting examples of such constructs are the constructs of SEQ ID NOS: 93 or 94.

In particular, it has been found in the invention (see the crystallography data referred to herein) that, when the Nanobodies present in a multivalent or multispecific protein or polypeptide of the invention bind to the particular epitope described above (which is the epitope of TNF1 and its humanized variants, as well as of TNF3 and its humanized variants) then preferably, the two (or more) anti-TNF Nanobodies present in such a protein or polypeptide should be linked in such a way that the distance between the N-terminus and the C-terminus of two anti-TNF Nanobodies present in such a protein or polypeptide should preferably be at least 50 Angstroms, and more preferably in the region of 55-200 Angstroms, and in particular in the region of 65-150 Angstroms (with the upper limit being less critical, and being chosen for reasons of convenience, e.g. with a view to expression/production of the protein); or more generally that said distance should be such that it allows the protein or polypeptide to undergo intramolecular binding to the TNF-trimer (i.e. instead of intermolecular binding). The distance between the N-terminus and the C-terminus of two anti-TNF Nanobodies can be determined by any suitable means, such as by crystallography or molecular modelling (as described herein). These techniques generally also make it possible to determine whether a specific multivalent or multispecific protein or polypeptide is capable of providing intramolecular modelling. Alternatively, the present invention also provides a simple experiment using size-exclusion chromatography (as described by Santora et al., Anal. Biochem., 299: 119-129) that can be used to determine whether a given protein or polypeptide of the invention will (predominantly) provide intramolecular binding to a TNF-trimer or (predominantly) intermolecular binding between two or more TNF-trimers. Thus, in one particular embodiment of the invention, a protein or polypeptide of the invention is preferably such that in this experiment, it predominantly or essentially exclusively leads to intramolecular binding However, as emphasized above, it should be noted that proteins or polypeptides of

the invention that operate via intermolecular binding of separate TNF-alpha molecules (e.g. trimers) are also within the scope of the present invention.

Thus, in another preferred aspect, the invention provides for a multivalent or multispecific protein or polypeptide that comprises at least two Nanobodies against TNF-alpha (and in particular of the TNF-alpha trimer), in which said Nanobodies are preferably directed to essentially the same epitope as Nanobody PMP1C2 (as mentioned herein), and in which said at least two Nanobodies are linked in such a way that the distance the distance between the N-terminus and the C-terminus of the at least two anti-TNF Nanobodies is such that the protein or polypeptide is capable of undergoing intramolecular binding (as described herein) with a TNF-trimer. Preferably, in such a protein or polypeptide, the distance between the N-terminus and the C-terminus of two anti-TNF Nanobodies is at least 50 Angstroms, and more preferably in the region of 55-200 Angstroms, and in particular in the region of 65-150 Angstroms.

In such a preferred protein or polypeptide, the two or more Nanobodies may be linked in any suitable fashion, as long as the preferred distance between the N-terminus and the C-terminus of the at least two anti-TNF Nanobodies can be achieved, and/or as long as the protein or polypeptide is capable of undergoing intramolecular binding (as described herein) with a TNF-trimer.

For example, in its simplest form, the at least two Nanobodies are directly linked via a suitable linker or spacer that provides for the preferred distance between the N-terminus and the C-terminus of the at least two anti-TNF Nanobodies and which may allow the protein or polypeptide to undergo intramolecular binding (as described herein) with a TNF-trimer. Suitable linkers are described herein, and may - for example and without limitation - comprise an amino acid sequence, which amino acid sequence preferably has a length of 14 amino acids, more preferably at least 17 amino acids, such as about 20-40 amino acid sequence (which, using an average distance of 3.5 Angstrom for one amino acid, corresponds to linker lengths of 49 Angstroms, 59.5 Angstroms and about 70 Angstroms, respectively; with the maximum amount of amino acids being calculated in the same way based on the distances mentioned above). Preferably, such an amino acid sequence should also be such that it allows the protein or polypeptide to undergo intramolecular binding (as described herein) with a TNF-trimer.

Thus, in another preferred aspect, the invention provides for a multivalent or multispecific protein or polypeptide that comprises at least two Nanobodies against TNF-

alpha (and in particular of the TNF-alpha trimer), in which said Nanobodies are preferably directed to essentially the same epitope as Nanobody PMP1C2 (as mentioned herein), and in which said at least two Nanobodies are directly linked to each other using a suitable linker or spacer such that the distance the distance between the N-terminus and the C-terminus of the at least two anti-TNF Nanobodies is such that the protein or polypeptide is capable of undergoing intramolecular binding (as described herein) with a TNF-trimer. Preferably, in such a protein or polypeptide, the distance between the N-terminus and the C-terminus of two anti-TNF Nanobodies (and thereby the preferred length of the linker or spacer) is at least 50 Angstroms, and more preferably in the region of 55-200 Angstroms, and in particular in the region of 65-150 Angstroms.

More preferably, in this preferred aspect, the linker or spacer is an amino acid sequence that comprises at least 14, preferably at least 17, more preferably at least 20 amino acids (with a non-critical upper limit chosen for reasons of convenience being abut 50, and preferably about 40 amino acids). In one preferred, but non-limiting embodiment, the linker essentially consists of glycine and serine residues (as further described below). For example, one suitable linker is the GS30 linker described herein, which comprises 30 amino acid residues.

In another embodiment, the at least two Nanobodies against TNF-alpha are linked to each other via another moiety (optionally via one or two linkers), such as another protein or polypeptide. In this embodiment, it may be desirable to have the preferred distance (i.e. as mentioned above) between the N-terminus and the C-terminus of the at least two anti-TNF Nanobodies, for example such that the protein or polypeptide can still undergo intramolecular binding (as described herein) with a TNF-trimer. In this embodiment, the at least two Nanobodies may be linked directly to the other moiety, or using a suitable linker or spacer, again as long as the preferred distance and/or desired intramolecular binding can still be achieved. The moiety may be any suitable moiety which does not detract (too much) from the binding of the protein or polypeptide to TNF and/or from the further desired biological or pharmacological properties of the protein or polypeptide. As such, the moiety may be essentially inactive or may be biologically active, and as such may or may not improve the desired properties of the protein or polypeptide and/or may confer one or more additional desired properties to the protein or polypeptide. For example, and without limitation, the moiety may improve the half-life of the protein or polypeptide, and/or may reduce its immunogenicity or improve any other desired property.

In one preferred embodiment, the moiety may be another Nanobody (including but not limited to a third Nanobody against TNF-alpha, although this is not necessary and usually less preferred), and in particular another Nanobody that improves the half-life of the protein or polypeptide, such as a Nanobody that is directed against a serum protein, for example against human serum albumin. Examples of such proteins and polypeptides are described herein.

Thus, in one embodiment, the invention relates to a multivalent multispecific construct comprising two or more immunoglobulin sequences (or suitable fragments thereof) that are each directed against epitopes on TNF-alpha (e.g. of the TNF-alpha trimer) that lie in and/or form part of the receptor binding site, and that are linked to each other via at least one immunoglobulin sequence that provides for increased half-life (and optionally via one or more suitable linkers), such that said polypeptide, upon binding to a TNF trimer, is capable inhibiting or reducing the TNF receptor crosslinking and/or the signal transduction that is mediated by said TNF trimer. Such a polypeptide may be such such that said firstmentioned two or more immunoglobulin sequences can each bind to a different receptor binding site on a TNF trimer.

In particular, in this embodiment, the polypeptide may comprise a trivalent bispecific Nanobody, that comprises two Nanobodies that are each directed against epitopes on TNF-alpha (and in particular of the TNF-alpha trimer) that lie in and/or form part of the receptor binding site, in which said Nanobodies are linked to each other via a third Nanobody that provides for an increased half-life (e.g. a Nanobody that is directed to a serum protein such as human serum albumin), in which each of the firstmentioned two Nanobodies may be directly linked to said third Nanobody or via one or more suitable linkers, such that said polypeptide, upon binding to a TNF trimer, is capable inhibiting or reducing the TNF receptor crosslinking and/or the signal transduction that is mediated by said TNF trimer. Such a polypeptide may be such that said firstmentioned two Nanobodies can each bind to a different receptor binding site on a TNF trimer. Again, some particularly preferred Nanobodies for use in this embodiment of the invention are PMP1C2 (TNF1, SEQ ID NO:52) and/or PMP5F10 (TNF3, SEQ ID NO: 60), as well as humanized and other variants thereof (as described herein); with PMP1C2 (TNF1, SEQ ID NO:52) and its humanized variants being particularly preferred; and the Nanobodies directed against human serum albumin described herein. Some preferred, but non-limiting constructs of this embodiment of the invention are TNF 24 (SEQ ID NO: 90), TNF 26

(SEQ ID NO: 92), TNF 27 (SEQ ID NO: 93), TNF 28 (SEQ ID NO: 94), TNF 60 (SEQ ID NO: 417) and TNF 62 (SEQ ID NO:418), of which TNF 60 is particularly preferred.

Thus, some preferred aspects of this embodiment of the invention relate to:

XIX) A protein or polypeptide that comprises or essentially consists of two or more immunoglobulin variable domains (or suitable fragments thereof) that are each directed against epitopes on TNF-alpha (and in particular of the TNF-alpha trimer) that lie in and/or form part of the receptor binding site(s) of the TNF trimer, such that said polypeptide, upon binding to a TNF trimer, is capable inhibiting or reducing the TNF receptor crosslinking that is mediated by said TNF trimer and/or the signal transduction that is mediated by such receptor crosslinking..

XX) A protein or polypeptide that comprises or essentially consists of two or more immunoglobulin variable domains (or suitable fragments thereof) that are each directed against epitopes on TNF-alpha (and in particular of the TNF-alpha trimer) that lie in and/or form part of the receptor binding site(s) of the TNF trimer, such that said polypeptide is capable intramolecular binding to at least two TNF receptor binding sites on a TNF trimer.

- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which said immunoglobulin variable domains are linked to each other in such a way that the protein or polypeptide is capable of simultaneously binding to two or more receptor binding sites on a single TNF trimer.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which said immunoglobulin variable domains are capable of binding to the same epitope as Nanobody TNF1 (SEQ ID NO: 52).
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which said immunoglobulin variable domains are capable of binding against the epitope within the TNF receptor binding site of the TNF trimer that at least comprises the following amino acid residues: Gln at position 88 in monomer A; Lys at position 90 in monomer A and Glu at position 146 in monomer B.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which said immunoglobulin variable domains are capable of binding against the epitope within the TNF receptor binding site of the TNF trimer that at least comprises the following amino acid residues: Gln at position 88 in monomer A; Lys at position 90 in monomer A and Glu at position 146 in monomer B; and that further

comprises at least one, preferably two or more, more preferably 5 or more, and preferably all or essentially all, of the following amino acid residues of TNF-alpha monomer A: Gly at position 24, Gln at position 25, Thr at position 72, His at position 73, Val at position 74, Leu at position 75, Thr at position 77, Thr at position 79, Ile at position 83, Thr at position 89, Val at position 91. Asn at position 92, Ile at position 97, Arg at position 131, Glu at position 135, Ile at position 136, Asn at position 137, Arg at position 138, Pro at position 139, Asp at position 140 and the following residues in monomer B: Pro at position 20, Arg at position 32, Lys at position 65, Lys at position 112, Tyr at position 115, Ala at position 145, Ser at position 147.

- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which said immunoglobulin variable domains are capable of binding against the epitope within the TNF receptor binding site of the TNF trimer that at least comprises the following amino acid residues: Gln at position 88 in monomer A; Lys at position 90 in monomer A and Glu at position 146 in monomer B; and that further comprises at least one, preferably two or more, more preferably 5 or more, and preferably all or essentially all, of the following amino acid residues of TNF-alpha monomer A Leu at position 75, Thr at position 77, Thr at position 79, Ile at position 80, Ser at position 81, Tyr at position 87, Thr at position 89, Val at position 91, Asn at position 92, Ser at position 95, Ile at position 97, Glu at position 135, Ile at position 136, Asn at position 137 and the following residues in monomer B: Ala at position 33, Ala at position 145, Ser at position 147.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the at least two immunoglobulin variable domains are linked in such a way that the distance between the N-terminus of the first immunoglobulin variable domain and the C-terminus of the second immunoglobulin variable domain present in such a protein or polypeptide is at least 50 Angstroms.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the distance between the N-terminus of the first immunoglobulin variable domain and the C-terminus of the second immunoglobulin variable domain is between 55-200 Angstroms
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the distance between the N-terminus of the first immunoglobulin variable

domain and the C-terminus of the second immunoglobulin variable domain is between 65-150 Angstroms

- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the first and second immunoglobulin variable domain are linked to each other via a linker or spacer.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the linker or spacer is an amino acid sequence.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the linker or spacer is comprises at least 14 amino acid residues.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the linker or spacer is comprises at least 17 - 50 amino acid residues.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the linker or spacer essentially consists of glycine and serine residues.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the linker or spacer is GS30 (SEQ ID NO: 69).
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the first and second immunoglobulin variable domain are linked to each other via another moiety.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which said other moiety is a protein or polypeptide moiety.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which said other moiety confers at least one desired property to the protein or polypeptide, or improves at least one desired property of the protein or polypeptide.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which said other moiety improves the half-life of the protein or polypeptide and/or reduces the immunogenicity of the protein or polypeptide.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which each of the first and second immunoglobulin variable domain are linked to said other moiety via a linker or spacer.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the linker or spacer is an amino acid sequence.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the linker or spacer essentially consists of glycine and serine residues.

- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the said other moiety is a Nanobody.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the other moiety is a Nanobody directed against human serum albumin.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the at least one Nanobody directed against human serum albumin is a humanized Nanobody.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the at least one Nanobody directed against human serum albumin is a humanized variant of the Nanobody ALB 1 (SEQ ID NO: 63).
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the at least one Nanobody directed against human serum albumin is a chosen from the group consisting of ALB 3 (SEQ ID NO: 87), ALB 4 (SEQ ID NO: 88), ALB 5 (SEQ ID NO: 89), ALB 6 (SEQ ID NO: 100), ALB 7 (SEQ ID NO: 101), ALB 8 (SEQ ID NO: 102) ALB 9 (SEQ ID NO: 103) and ALB 10 (SEQ ID NO: 104).
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the at least one Nanobody directed against human serum albumin is ALB 8.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the first and second immunoglobulin variable domains are Nanobodies.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the first and second immunoglobulin variable domains are Nanobodies with a K_{off} rate for TNF of better than 2.10^{-3} (1/s), preferably better than 1.10^{-3} (1/s); or a humanized variant of such a Nanobody.,
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the first and second immunoglobulin variable domains are Nanobodies with an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than the EC50 value of Nanobody VHH 3E (SEQ ID NO:4) of WO 04/041862 in the same assay; or a humanized variant of such a Nanobody..
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the first and second immunoglobulin variable domains are Nanobodies with an EC50 value in the cell-based assay using KYM cells described in Example 1,

under 3), of WO 04/041862 that is better than 12nM; or a humanized variant of such a Nanobody..

- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the first and second immunoglobulin variable domains are Nanobodies with an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 5nM; or a humanized variant of such a Nanobody..
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the first and second immunoglobulin variable domains are Nanobodies with an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 3nM; or a humanized variant of such a Nanobody..
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the first and second immunoglobulin variable domains are Nanobodies in accordance with any one of XIX) to XX) above , which are humanized;

with some particularly preferred aspects of this embodiment being:

- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the first and second immunoglobulin variable domains are GLEW-class Nanobodies.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the first and second immunoglobulin variable domains are Nanobodies with an arginine residue (R) at position 103.
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the first and second immunoglobulin variable domains are GLEW-class Nanobodies with an arginine residue (R) at position 103..
- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the first and second immunoglobulin variable domains are Nanobodies with at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the amino acid sequences of SEQ ID NO's 52 (TNF1), 76 (TNF13), 77 (TNF14), 95 (TNF29) or 96 (TNF30)
- A protein or polypeptide in accordance with any one of XIX) to XX) above, in which the first and second immunoglobulin variable domains are Nanobodies as

described for the proteins or polypeptides in accordance with any one of XIX) to XX) in which:

a) CDR1 comprises:

- the amino acid sequence DYWMY; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence DYWMY; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence DYWMY;

and

b) CDR2 comprises:

- the amino acid sequence EINTNGLITKYPDSVKG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence EINTNGLITKYPDSVKG; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence EINTNGLITKYPDSVKG;

and

c) CDR3 comprises:

- the amino acid sequence SPSGFN; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence SPSGFN; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence SPSGFN.

- A protein or polypeptide in accordance with any one of XIX) to XX) above , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) in which CDR1 comprises the amino acid sequence DYWMY.
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for

the proteins or polypeptides in accordance with any one of XIX) to XX) in which CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG.

- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) in which CDR3 comprises the amino acid sequence SPSGFN
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) in which:
  - CDR1 comprises the amino acid sequence DYWMY; and CDR3 comprises the amino acid sequence SPSGFN; or
  - CDR1 comprises the amino acid sequence DYWMY; and CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG; or
  - CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG; and CDR3 comprises the amino acid sequence SPSGFN
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) in which CDR1 comprises the amino acid sequence DYWMY; and CDR3 comprises the amino acid sequence SPSGFN.
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) in which CDR1 comprises the amino acid sequence DYWMY; CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG and CDR3 comprises the amino acid sequence SPSGFN.
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) in which
  a) CDR1 is:
     - the amino acid sequence DYWMY; or

- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence DYWMY; or
- an amino acid sequences that has 2 or only 1 amino acid difference with the amino acid sequence DYWMY;

and in which:

b) CDR2 is:

- the amino acid sequence EINTNGLITKYPDSVKG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence EINTNGLITKYPDSVKG; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence EINTNGLITKYPDSVKG;

and in which

c) CDR3 is:

- the amino acid sequence SPSGFN; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence SPSGFN; or
- an amino acid sequences that has 2 or only 1 amino acid difference with the amino acid sequence SPSGFN.

- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) in which CDR1 is the amino acid sequence DYWMY.
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) in which CDR2 is the amino acid sequence EINTNGLITKYPDSVKG.
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for

the proteins or polypeptides in accordance with any one of XIX) to XX) in which CDR3 is the amino acid sequence SPSGFN

- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) in which:
  - CDR1 is the amino acid sequence DYWMY; and CDR3 is the amino acid sequence SPSGFN; or
  - CDR1 is the amino acid sequence DYWMY; and CDR2 is the amino acid sequence EINTNGLITKYPDSVKG; or
  - CDR2 is the amino acid sequence EINTNGLITKYPDSVKG; and CDR3 is the amino acid sequence SPSGFN
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) in which CDR1 is the amino acid sequence DYWMY; and CDR3 is the amino acid sequence SPSGFN.
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) in which CDR1 is the amino acid sequence DYWMY; CDR2 is the amino acid sequence EINTNGLITKYPDSVKG and CDR3 is the amino acid sequence SPSGFN.
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) in which
  - any amino acid substitution is preferably a conservative amino acid substitution; and/or
  - said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s).
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are chosen from the group consisting of the Nanobody TNF 1 (SEQ ID NO: 52) and humanized variants of the Nanobody TNF 1 (SEQ ID NO: 52).

- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are chosen from the group consisting of TNF 13 (SEQ ID NO: 76), TNF 14 (SEQ ID NO: 77), TNF 29 (SEQ ID NO: 95) and TNF 30 (SEQ ID NO:96).
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are TNF 30 (SEQ ID NO:96);

and with some particularly preferred aspects of this embodiment being:

- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are KERE-class Nanobodies.
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies with at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the amino acid sequences of SEQ ID NO's 50 (TNF3), 83 (TNF20), 85 (TNF21), 85 (TNF22), 96 (TNF23) or 98 (TNF33).
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) in which:

a) CDR1 comprises:

- the amino acid sequence NYYMG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence NYYMG; or
- an amino acid sequences that has 2 or only 1 amino acid difference with the amino acid sequence NYYMG;

and

b) CDR2 comprises:

- the amino acid sequence NISWRGYNIYYKDSVKG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence NISWRGYNIYYKDSVKG; or

- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence NISWRGYNIYYKDSVKG;

and

c) CDR3 comprises:

- the amino acid sequence SILPLSDDPGWNTY; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence SILPLSDDPGWNTY; or
- an amino acid sequences that has 2 or only 1 amino acid difference with the amino acid sequence SILPLSDDPGWNTY.

- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) in which CDR1 comprises the amino acid sequence NYYMG.
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) in which CDR2 comprises the amino acid sequence NISWRGYNIYYKDSVKG.
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) in which CDR3 comprises the amino acid sequence SILPLSDDPGWNTY.
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) , in which:
  - CDR1 comprises the amino acid sequence NYYMG; and CDR3 comprises the amino acid sequence SILPLSDDPGWNTY; or
  - CDR1 comprises the amino acid sequence NYYMG; and CDR2 comprises the amino acid sequence NISWRGYNIYYKDSVKG; or
  - CDR2 comprises the amino acid sequence NISWRGYNIYYKDSVKG; and CDR3 comprises the amino acid sequence SILPLSDDPGWNTY.

- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) , in which CDR1 comprises the amino acid sequence NYYMG; CDR2 comprises the amino acid sequence SILPLSDDPGWNTY and CDR3 comprises the amino acid sequence ILPLSDDPGWNTY.
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) , in which

a) CDR1 is:

- the amino acid sequence NYYMG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence NYYMG; or
- an amino acid sequences that has 2 or only 1 amino acid difference with the amino acid sequence NYYMG;

and

b) CDR2 is:

- the amino acid sequence NISWRGYNIYYKDSVKG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence NISWRGYNIYYKDSVKG; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence NISWRGYNIYYKDSVKG;

and

c) CDR3 is:

- the amino acid sequence SILPLSDDPGWNTY; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence SILPLSDDPGWNTY; or

- an amino acid sequences that has 2 or only 1 amino acid difference with the amino acid sequence SILPLSDDPGWNTY.

- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) , in which CDR1 is the amino acid sequence NYYMG.
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) , in which CDR2 is the amino acid sequence NISWRGYNIYYKDSVKG.
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) , in which CDR3 is the amino acid sequence SILPLSDDPGWNTY.
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) , in which:
  - CDR1 is the amino acid sequence NYYMG; and CDR3 is the amino acid sequence SILPLSDDPGWNTY; or
  - CDR1 is the amino acid sequence NYYMG; and CDR2 is the amino acid sequence NISWRGYNIYYKDSVKG; or
  - CDR2 is the amino acid sequence NISWRGYNIYYKDSVKG; and CDR3 is the amino acid sequence SILPLSDDPGWNTY.
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) , in which CDR1 is the amino acid sequence NYYMG; CDR2 is the amino acid sequence SILPLSDDPGWNTY and CDR3 is the amino acid sequence ILPLSDDPGWNTY.
- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are Nanobodies as described for the proteins or polypeptides in accordance with any one of XIX) to XX) , in which
  - any amino acid substitution is preferably a conservative amino acid substitution; and/or

- said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s).

- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are chosen from the group consisting of the Nanobody TNF 3 (SEQ ID NO: 60) and humanized variants of the Nanobody TNF 3 (SEQ ID NO: 60).

- A protein or polypeptide in accordance with any one of XIX) to XX) , in which the first and second immunoglobulin variable domains are chosen from the group consisting of 83 TNF20 (SEQ ID NO:83), TNF21 (SEQ ID NO: 84), TNF 22 (SEQ ID NO: 85), TNF23 (SEQ ID NO: 86) or TNF33 (SEQ ID NO: 99).

It should be noted that when a protein or polypeptide is mentioned above as being "*in accordance with any one of XIX) to XX) above*", it is at least according to one of XIX) to XX), and may be according to both XIX) and XX), and may also include any one or more of the other aspects that are indicated as being "in accordance with any one of XIX) to XX) above".

However, it should be noted that the invention is not limited to any specific mechanism of action or hypothesis. In particular, it has been found that the monovalent Nanobodies of the invention may be also active in the assays and models described herein, which confirms that intramolecular binding of he TNF trimer, although preferred in one specific embodiment of the invention, is not required to obtain the desired action and effect of the Nanobodies, proteins and polypeptides described herein. Similarly, it is also encompassed within the scope of the invention that the proteins and polypeptides described herein achieve their desired action via any appropriate mechanism (i.e. by intramolecular binding, intermolecular binding or even by binding to monomeric TNF, thus inhibiting the formation of TNF trimers).

It is also within the scope of the invention to use parts, fragments, analogs, mutants, variants, alleles and/or derivatives of the Nanobodies and polypeptides of the invention, and/or to use proteins or polypeptides comprising or essentially consisting of the same, as long as these are suitable for the uses envisaged herein. Such parts, fragments, analogs, mutants, variants, alleles, derivatives, proteins and/or polypeptides will be described in the further description herein.

In another aspect, the invention relates to a Nanobody (as defined herein), against TNF-alpha, which consist of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), in which:

(i) CDR1 is an amino acid sequence chosen from the group consisting of the CDR1 sequences of SEQ ID NOS: 15 to 21 or from the group consisting of the CDR1 sequences of SEQ ID NOS: 164 to 197;

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with at least one of the CDR1 sequences from the group consisting of SEQ ID NOS: 15 to 21 or with at least one of the CDR1 sequences from the group consisting of SEQ ID NOS: 164 to 197, in which

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or from the group consisting of amino acid sequences that have 2 or only 1 "amino acid difference(s)" (as defined herein) with at least one of the CDR1 sequences from the group consisting of SEQ ID NOS: 15 to 21 or with at least one of the CDR1 sequences from the group consisting of SEQ ID NOS: 164 to 197, in which:

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and in which:

(ii) CDR2 is an amino acid sequence chosen from the group consisting of the CDR2 sequences of SEQ ID NOS: 22 to 28 or from the group consisting of the CDR2 sequences of SEQ ID NOS: 232 to 265;

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with at least one of the CDR2 sequences

from the group consisting of SEQ ID NOS: 22 to 28 or with at least one of the CDR2 sequences from the group consisting of SEQ ID NOS: 232 to 265, in which

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with at least one of the CDR2 sequences from the group consisting of SEQ ID NOS: 22 to 28 or with at least one of the CDR2 sequences from the group consisting of SEQ ID NOS: 232 to 265, in which:

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and in which:

(iii) CDR3 is an amino acid sequence chosen from the group consisting of the CDR3 sequences of SEQ ID NOS: 29 to 33 or from the group consisting of the CDR3 sequences of SEQ ID NOS: 300-333;

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with at least one of the CDR3 sequences from the group consisting of SEQ ID NOS: 29 to 33 or with at least one of the CDR3 sequences from the group consisting of SEQ ID NOS: 300-333, in which:

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) or with at least one of the CDR3 sequences from the group consisting of SEQ ID NOS: 300-333, in which:

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

or from the group consisting of the CDR3 sequences of SEQ ID NOS: 34 and 35 or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with at least one of the CDR3 sequences of SEQ ID NOS: 34 and 35, in which:

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with at least one of the CDR3 sequences of SEQ ID NOS: 34 and 35, in which:

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s).

The Nanobodies against TNF-alpha as described above and as further described hereinbelow are also referred to herein as Nanobodies of the invention.

Of the Nanobodies of the invention, Nanobodies comprising one or more of the CDR's explicitly listed above are particularly preferred; Nanobodies comprising two or more of the CDR's explicitly listed above are more particularly preferred; and Nanobodies comprising three of the CDR's explicitly listed above are most particularly preferred.

Some particularly preferred, but non-limiting combinations of CDR sequences can be seen in Table I below, which lists the CDR's and framework sequences that are present in a number of preferred (but non-limiting) Nanobodies of the invention. As will be clear to the skilled person, a combination of CDR1, CDR2 and CDR3 sequences that occur in the same clone (i.e. CDR1, CDR2 and CDR3 sequences which are mentioned on the same line in Table I) will usually be preferred (although the invention in its broadest sense is not limited thereto, and also comprises other suitable combinations of the CDR sequences mentioned in Table I).

Also, in the Nanobodies of the invention that comprise the combinations of CDR's mentioned in Table I, each CDR can be replaced by a CDR chosen from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with the mentioned CDR's; in which

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or chosen from the group consisting of amino acid sequences that have 3, 2 or only 1 (as indicated in the preceding paragraph) "amino acid difference(s)" (as defined herein) with the mentioned CDR(s) one of the above amino acid sequences, in which:

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s).

However, as will be clear to the skilled person, the (combinations of) CDR sequences mentioned in Table I will generally be preferred.

Table I: Preferred combinations of framework and CDR sequences

Clone	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
PMP1C2 (TNF1)	130	QVQLVESGGGLVQPG GSLRLSCAASGFTFS	164	DYWMY	198	WVRQAPGKG LEWVS	233	EINTNGLITKYP DSVKG	266	RFTISRDNAKNTLYLQMNSL KPEDTALYYCAR	300	SPSGFN	334	RGQGT QVTVSS
PMP1G11	131	QVQLQESGGGMVQPG GSLRLSCAASGFDFG	165	VSWMY	199	WVRQAPGKG LEWVS	233	EINTNGLITKYP DSVKG	266	RFTISRDNAKTTLYLQMNSL KPEDTALYYCAR	301	SPSGSF	335	RGQGT QVTVSS
PMP1H6	132	EVQLVESGGGLVQPG GSLRLSCATSGFDFS	166	VSWMY	200	WVRQAPGKG LEWVS	234	EINTNGLITKYV DSVKG	268	RFTISRDNAKNTLYLQMDSL IPEDTALYYCAR	302	SPSGSF	336	RGQGT QVTVSS
PMP1G5 (TNF2)	133	QVQLVESGGGLVQAG GSLRLSCAASGRTFS	167	EPSGYT YTIG	201	WFRQAPGKE REFVA	235	RIYWSSGLTYY ADSVKG	269	RFTISRDIAKNTVDLLMNSL KPEDTAVYYCAA	303	RDGIPTSRSV GSYNY	337	WGQGT QVTVSS
PMP1H2	134	QVKLEESGGGLVQPG DSLRLSCAASGRTFS	168	DYSGYT YTVG	202	WFRQAPGKE REFVA	236	RIYWSSGNTYY ADSVKG	270	RFTISRDIAKNTVDLLMNNL EPEDTAVYYCAA	304	RDGIPTSRSV ESYNY	338	WGQGT QVTVSS
PMP3G2	135	AVQLVESGGGLVQPG DSLRLSCAASGRTFS	169	DYSGYT YTVG	203	WFRQAPGKE REFVA	237	RIYWSSGNTYY ADSVKG	271	RFTISRDIAKNTVDLLMNNL EPEDTAVYYCAA	305	RDGIPTSRSV ESYNY	339	WGQGT QVTVSS
PMP1D2	136	AVQLVDSGGGLVQAG GSLRLSCAASGRTFS	170	AHSVYT MG	204	WFRQAPGKE REFVA	238	RIYWSSANTYY ADSVKG	272	RFTISRDNAKNTVDLLMNCL KPEDTAVYYCAA	306	RDGIPTSRSV EAYNY	340	WGQGT QVTVSS

Table I (continued):

Clone	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
PMP3D10	137	QVQLVESGGGLVQAGGSLSLSCAASGRSFT	171	GYYMG	205	WFRQAPGKERQLLA	239	SISWRGDNTYYKESVKG	273	RFTISRDDAKNTIYLQMNSLKPEDTAVYYCAA	307	SILPLSDDPGWNTN	341	WGQGTQVTVSS
PMP5F10 (TNF3)	138	EVQLVESGGGLVQAGGSLSLSCSASGRSLS	172	NYYMG	206	WFRQAPGKERELLG	240	NISWRGYNIYYKDSVKG	274	RFTISRDDAKNTIYLQMNRLKPEDTAVYYCAA	308	SILPLSDDPGWNTY	342	WGQGTQVTVSS
NC55TNF_S1C4	139	EVQLVESGGGLVQAGDSLRLSCAASQIIFG	173	SHVAA	207	WFRQAPGREREFVA	241	EIRPSGDFGPEGEFEHVTASLKG	275	RFTIAKNSVDNTVYLQMNSLKPEDTAVYYCAA	309	APYRGGRDYRWEYEYEY	343	WGQGTQVTVSS
NC55TNF_S1C3	140	EVQLVESGGGLVQPGGSLRLSCKNAGSTSN	174	AYATG	208	WFRRAPGKEREFVA	242	GIQWSGGDAFYRNSVKG	276	RFRITRDPDNTVYLQMNDLKPEDTAIYYCAQ	310	KLSPYYNDFDSSNYEY	344	WGQGTQVTVSS
NC55TNF_S2C1	141	EVQLVESGGDLVQPGGSLRLSCAVSGQLFS	175	TNDVG	209	WYRRAPGKQRELVA	243	TITDDGTTDYGDDVKG	277	RFVISREGEMVYLEMNSLKPEDTAVYYCNI	311	NRLRSTWGIRYDV	345	WGQGTQVTVSS
NC55TNF_S2C5	142	EVQLVESGGGLVQPGGSLRLSCVVSGFTFS	176	TTSMT	210	WVRQAPGKFEEWVS	244	FINSDGSSTTYADSVKG	278	RFTISRDNAKNTLYLQMNSLKPEDTAMYYCGR	312	RGYGRD	346	RSKGIQVTVAS
NC55TNF_S3C7	143	EVQLVESGGGTVQAGDSLRLSCAASGRSFS	177	SVAMG	211	WFRQAPGKQREFLA	245	GVGYDGSSIRYAESVKG	279	RFTIARGNRESTVFLQMNENLKPEDTAVYFCTA	313	EPIGAYEGLWTY	347	WGQGTQVTVSS

Table I (continued):

Clone	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
NC55TNF_S3C1	14	TNF-ALPHAVESGGGLMQPGGSLKLSCAASGFMFS	178	DSAMG	21	WFRQAPGKEREFVA	46	TISWNGGSSSYADFVKG	280	RFTISRDNAKNTVYLQMNGLTPQDTAIYYCAG	314	SYSNGNPHRFSQYQY	348	WGQGTQVTVSS
NC55TNF_BMP1B2	145	EVQLVESGGGLVQAGGSLRLSCAASGRTFG	179	TYAMG	31	WFRQAPGKEREFVA	47	AISWGGGSIVYAESAKG	281	RFTISRDNAKXTMYLQMDSLKPEDTAVYYCAA	315	ANNIATLRQGS	349	WGQGTQVTVSS
NC55TNF_BMP1D2	146	EVQLVESGGELVQAGGSLKLSCTASGRNFV	180	TYAMS	41	WFRRAPGKEREFVA	48	SISWSGDTTYYSNSVKG	282	RFTVSRDNGKNTAYLRMNSLKPEDTADYYCAV	316	VQVIDPSWSGVNLDDYDY	350	LGSGTQVTVSS
NC55TNF_BMP1E2	147	EVQLVESGGRLVQPGGSLRLSCKNAGSTSN	181	AYATG	51	WFRRAPGKEREFVA	49	GIQWSGGDAFYRNSVKG	283	RFRITRDPDNTVYLQMNDLKPEDTAIYYCAQ	317	KLSPYYNDFDSSNYEY	351	WGQGTQVTVSS
NC55TNF_BMP1G2	148	EVQLVESGGGLVQPGGSLRLSCAASATISS	182	IVMLG	61	WYRQAPGKQREWVA	50	SITIGSRTNYADSVKG	284	RFTISRDNAKNTVYLQMNSLKPEDTAVYFCNA	318	VPPRDDY	352	WGQGTQVTVSS
NC55TNF_BMP2A2	149	EVQLVESGGGLVQAGGSLRLSCAASGQTSS	183	SYDMG	71	WFRQAPGEGREFVA	51	RISGSDGSTYYSDRAKD	285	RFTISRDNTKNMVYLQMDRLKPDDTAVYYCRV	319	PRYENQWSSYDY	353	WGQGTQVTVSS
NC55TNF_BMP2C2	150	EVQLVESGGGLVQPGGSLRLSCAASGSTFS	184	TYDMS	81	WVRQAPGKGLEWVS	52	GIDSGGGSPMYVDSVKG	286	RFTVSRDNAKNTLYLQMNSLKPEDTAVYYCAK	320	FSTGADGGSWYWSYGMDS	354	WGKGTQVTVSS

Table I (continued):

Clone	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
NC55TNF_BMP2F2	15	EVQLVESGGGLVQAG	18	RYNMA	21	WFRQAPGK	25	RVDVSGGNT	28	RFTVSRINGKNAMYLQMNNL	32	GGWGTTQYDY	35	WGQG TQVTV
	11	DSLRLSCEASERSSN	59		91	EREFLA	33	LYGDSVKD	71	KPEDTAIYYCAA	11	DY	55	SS
NC55TNF_NC10	15	EVQLVESGGGLVQPG	18	FSAYSMT	22	WVRQAPGK	25	FINSDGSSTT	28	RFKISRDNAKKTLYLQMNSL	32		35	RGQGT QVTVS
	12	GSLRLSCVCVSSGCT	66		02	AEEWVS	44	YADSVNG	88	GPEDTAMYYCQR	22	RGYALD	66	S
NC55TNF_NC11	15	EVQLVESGGGLVQAG	18	KNAMG	22	WFRQPPGK	25	SIKWNGNNT	28	RFTISRGNAKNTENTVSLQM	32	DSSHYSYVYSK	35	WGQG TQVTV
	13	DSLTLSCASSGRGFY	77		11	EREFVA	55	YYADSVRG	99	NSLKPEDTADYYCAA	33	AYEYDY	77	SS
NC55TNF_NC1	15	EVQLVESGGGLVQPG	18	ASSMA	22	WVRQAPGK	25	FINSDGSSTT	29	RFTISRDNAKNTLYLQMNSL	32		35	RSQGI QVTVS
	14	GSLRLSCVFSGFAFS	88		22	YEEWVS	66	YADSVQG	00	KSEDTAMYYCGR	44	RGYGRD	88	S
NC55TNF_NC2	15	EVQLVESGGGLVQAG	18	SYAMG	22	WFRQAPGK	25	AISWSGTITN	29	RFTISRDNGKNTVHLQMNSL	32	VQPYSGGDYY	35	WGXG TQVTV
	15	GSLRLSCAASGRTFS	99		33	EREFVA	77	YADSVKG	11	KPEDTAVYHCAV	55	TGVEEYDY	99	SS
NC55TNF_NC3	15	EVQLVESGGGLVQPG	19	ATSMT	22	WVRQAPGK	25	FINSDGSSTT	29	RFTISRDNAKNTLYLQMDDL	32		36	RSRGI QVTVS
	16	GSLRLSCVVSGFTFS	00		44	AEEWVS	88	YADSVKG	22	QSEDTAMYYCGR	66	RGYGRD	00	S
NC55TNF_NC5	15	EVQLVESGGGLVQAG	19	NYDVG	22	WFRQAPGE	25	RISGSGDST	29	RFTISRDNAKNTVYLQMNSL	32	ARYNGTWSSN	36	WGQG TQVTV
	17	GSLRLSCAASGGAFS	11		55	GREIVA	99	YSSNRAKG	33	KREDTAVYYCRA	77	DY	11	SS

Table I (continued):

Clone	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
NC55TNF_NC6	15 18	EVQLVESGGGLVQPGGSLRLSCECVSSGCT	19 2	FSAYSMT	22 6	WVRQAPGKAEEFVS	26 0	FINSDGSSTTYANSVNG	29 4	RFKISRDNAKKTLYLQMNSLGPEDTAMYYCQR	32 8	RGYALD	36 2	RGQGTQVTVSS
NC55TNF_NC7	15 19	QVQLVESGGGLVQAGGSLRLSCTASGQTSS	19 3	TADMG	22 7	WFRQPPGKGREFVA	26 1	RISGIDGTTYYDEPVKG	29 5	RFTISRDKAQNTVYLQMDSLKPEDTAVYYCRS	32 9	PRYADQWSAYDY	36 3	WGQGTQVTVSS
NC55TNF_NC8	16 0	EVQLVESGGGLVQPGGSLRLSCVVSGFTFS	19 4	TTSMT	22 8	WVRQAPGKFEEWVS	26 2	FINSDGSSTTYADSVKG	29 6	RFTISRDNAKNTLYLQMNSLKPEDTAMYYCGR	33 0	RGYGRD	36 4	RSKGIQVTVSS
NC55TNF_S2C2	16 1	EVQLVESGGGLVQPGGSLRLSCVASASGVK	19 5	VNDMG	22 9	WYRQAPGKERELVA	26 3	TITDDGRTNYEDFAKG	29 7	RFTISRDNAKNTVYLQMNSLLPEDTAVYYCNA	33 1	RTYWAHLPTY	36 5	WGQGTQVTVSS
NC55TNF_S1C6	16 2	EVQLVESGGGLVQAGGSLRLSCAASGRSFG	19 6	SVAMG	23 0	WFRQAPGKEREFVA	26 4	AIGYDGNSIRYGDSVKG	29 8	RFTISRDNIKNTMYLEMENLNADDTARYLCAA	33 2	EPLARYEGLWTY	36 6	WGQGTQVTVSS
NC55TNF_S3C2	16 3	EVQLVESGGGLVQAGASLRLSCTTSTRTN	19 7	DRFNMA	23 1	WFHQAPGKDREFVS	26 5	RIDVAGYNTAYGDFVKG	29 9	RFTVSRDSAENTVVLQMNSLRPEDTGVYYCAA	33 3	GGWGISQSDYDL	36 7	WGQGTQVTVSS

Notes to Table I:

- ID refers to the SEQ ID NO's in the attached sequence listing

- For CDR1: SEQ ID NO: 164 corresponds to SEQ ID NO: 15, SEQ ID NO: 167 corresponds to SEQ ID NO: 16; SEQ ID NO: 172 corresponds to SEQ ID NO: 17, SEQ ID NOS: 165 and 166 correspond to SEQ ID NO: 18, SEQ ID NO: 170 corresponds to SEQ ID NO: 19, SEQ ID NO: 171 corresponds to SEQ ID NO: 20, and SEQ ID NOS: 168 and 169 correspond to SEQ ID NO: 21.

- For CDR2: SEQ ID NOS: 232 and 233 correspond to SEQ ID NO: 22, SEQ ID NO: 235 corresponds to SEQ ID NO: 23, SEQ ID NO: 240 corresponds to SEQ ID NO: 24, SEQ ID NO: 234 corresponds to SEQ ID NO: 25, SEQ ID NOS: 236 and 237 correspond to SEQ ID NO: 26, SEQ ID NO: 238 corresponds to SEQ ID NO: 27, and SEQ ID NO: 239 corresponds to SEQ ID NO: 28.

- For CDR3: SEQ ID NO: 303 corresponds to SEQ ID NO: 29, SEQ ID NO: 308 corresponds to SEQ ID NO: 30, SEQ ID NO: 306 corresponds to SEQ ID NO: 31, SEQ ID NO: 307 corresponds to SEQ ID NO: 32, SEQ ID NOS: 304 and 305 corresponds to SEQ ID NO: 33, SEQ ID NO: 300 corresponds to SEQ ID NO: 34, and SEQ ID NOS: 301 and 302 correspond to SEQ ID NO: 35.

Thus, in the Nanobodies of the invention, at least one of the CDR1, CDR2 and CDR3 sequences present is suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table I; or from the group of CDR1, CDR2 and CDR3 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% "sequence identity" (as defined herein) with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table I; and/or from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table I. In this context, by "suitably chosen" is meant that, as applicable, a CDR1 sequence is chosen from suitable CDR1 sequences (i.e. as defined herein), a CDR2 sequence is chosen from suitable CDR2 sequences (i.e. as defined herein) and a CDR3 sequence is chosen from suitable CDR3 sequences (i.e. as defined herein), respectively.

In particular, in the Nanobodies of the invention, at least the CDR3 sequence present is suitably chosen from the group consisting of the CDR3 sequences listed in Table I or from the group of CDR3 sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR3 sequences listed in Table I; and/or from the group consisting of the CDR3 sequences that have 3, 2 or only 1 amino acid difference(s) with at least one of the CDR3 sequences listed in Table I.

Preferably, in the Nanobodies of the invention, at least two of the CDR1, CDR2 and CDR3 sequences present are suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table I or from the group consisting of CDR1, CDR2 and CDR3 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table I; and/or from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, that have 3, 2 or only 1 "amino acid difference(s)" with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table I.

In particular, in the Nanobodies of the invention, at least the CDR3 sequence present is suitably chosen from the group consisting of the CDR3 sequences listed in Table I or from the group of CDR3 sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity

with at least one of the CDR3 sequences listed in Table I, respectively; and at least one of the CDR1 and CDR2 sequences present is suitably chosen from the group consisting of the CDR1 and CDR2 sequences, respectively, listed in Table I or from the group of CDR1 and CDR2 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR1 and CDR2 sequences, respectively, listed in Table I; and/or from the group consisting of the CDR1 and CDR2 sequences, respectively, that have 3, 2 or only 1 amino acid difference(s) with at least one of the CDR1 and CDR2 sequences, respectively, listed in Table I.

Most preferably, in the Nanobodies of the invention, all three CDR1, CDR2 and CDR3 sequences present are suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table I or from the group of CDR1, CDR2 and CDR3 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table I; and/or from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, that have 3, 2 or only 1 amino acid difference(s) with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table I.

Even more preferably, in the Nanobodies of the invention, at least one of the CDR1, CDR2 and CDR3 sequences present is suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table I. Preferably, in this embodiment, at least one or preferably both of the other two CDR sequences present are suitably chosen from CDR sequences that that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the corresponding CDR sequences, respectively, listed in Table I; and/or from the group consisting of the CDR sequences that have 3, 2 or only 1 amino acid difference(s) with at least one of the corresponding sequences, respectively, listed in Table I.

In particular, in the Nanobodies of the invention, at least the CDR3 sequence present is suitably chosen from the group consisting of the CDR3 listed in Table I. Preferably, in this embodiment, at least one and preferably both of the CDR1 and CDR2 sequences present are suitably chosen from the groups of CDR1 and CDR2 sequences, respectively, that that have at least 80%, preferably at least 90%, more preferably at least

95%, even more preferably at least 99% sequence identity with the CDR1 and CDR2 sequences, respectively, listed in listed in Table I; and/or from the group consisting of the CDR1 and CDR2 sequences, respectively, that have 3, 2 or only 1 amino acid difference(s) with at least one of the CDR1 and CDR2 sequences, respectively, listed in Table I.

Even more preferably, in the Nanobodies of the invention, at least two of the CDR1, CDR2 and CDR3 sequences present are suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table I. Preferably, in this embodiment, the remaining CDR sequence present are suitably chosen from the group of CDR sequences that that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the corresponding CDR sequences listed in Table I; and/or from the group consisting of CDR sequences that have 3, 2 or only 1 amino acid difference(s) with at least one of the corresponding sequences listed in Table I.

In particular, in the Nanobodies of the invention, at least the CDR3 sequence is suitably chosen from the group consisting of the CDR3 sequences listed in Table I, and either the CDR1 sequence or the CDR2 sequence is suitably chosen from the group consisting of the CDR1 and CDR2 sequences, respectively, listed in Table I. Preferably, in this embodiment, the remaining CDR sequence present are suitably chosen from the group of CDR sequences that that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the corresponding CDR sequences listed in Table I; and/or from the group consisting of CDR sequences that have 3, 2 or only 1 amino acid difference(s) with the corresponding CDR sequences listed in Table I.

Even more preferably, in the Nanobodies of the invention, all three CDR1, CDR2 and CDR3 sequences present are suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table I.

Also, generally, the combinations of CDR's listed in Table I (i.e. those mentioned on the same line in Table I) are preferred. Thus, it is generally preferred that, when a CDR in a Nanobody of the invention is a CDR sequence mentioned in Table I or is suitably chosen from the group of CDR sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with a CDR sequence listed in Table I; and/or from the group consisting of CDR sequences that have

3, 2 or only 1 amino acid difference(s) with a CDR sequence listed in Table I, that at least one and preferably both of the other CDR's are suitably chosen from the CDR sequences that belong to the same combination in Table I (i.e. mentioned on the same line in Table I) or are suitably chosen from the group of CDR sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the CDR sequence(s) belonging to the same combination and/or from the group consisting of CDR sequences that have 3, 2 or only 1 amino acid difference(s) with the CDR sequence(s) belonging to the same combination.The other preferences indicated in the above paragraphs also apply to the combinations of CDR's mentioned in Table I.

Thus, by means of non-limiting examples, a Nanobody of the invention can for example comprise a CDR1 sequence that has more than 80 % sequence identity with one of the CDR1 sequences mentioned in Table I, a CDR2 sequence that has 3, 2 or 1 amino acid difference with one of the CDR2 sequences mentioned in Table I (but belonging to a different combination), and a CDR3 sequence.

Some preferred Nanobodies of the invention may for example comprise: (1) a CDR1 sequence that has more than 80 % sequence identity with one of the CDR1 sequences mentioned in Table I; a CDR2 sequence that has 3, 2 or 1 amino acid difference with one of the CDR2 sequences mentioned in Table I (but belonging to a different combination); and a CDR3 sequence that has more than 80 % sequence identity with one of the CDR3 sequences mentioned in Table I (but belonging to a different combination); or (2) a CDR1 sequence that has more than 80 % sequence identity with one of the CDR1 sequences mentioned in Table I; a CDR2 sequence, and one of the CDR3 sequences listed in Table I; or (3) a CDR1 sequence; a CDR2 sequence that has more than 80% sequence identity with one of the CDR2 sequence listed in Table I; and a CDR3 sequence that has 3, 2 or 1 amino acid differences with the CDR3 sequence mentioned in Table I that belongs to the same combination as the CDR2 sequence.

Some particularly preferred Nanobodies of the invention may for example comprise: (1) a CDR1 sequence that has more than 80 % sequence identity with one of the CDR1 sequences mentioned in Table I; a CDR2 sequence that has 3, 2 or 1 amino acid difference with the CDR2 sequence mentioned in Table I that belongs to the same combination; and a CDR3 sequence that has more than 80 % sequence identity with the CDR3 sequence mentioned in Table I that belongs to the same combination; (2) a CDR1

sequence; a CDR 2 listed in Table I and a CDR3 sequence listed in Table I (in which the CDR2 sequence and CDR3 sequence may belong to different combinations).

Some even more preferred Nanobodies of the invention may for example comprise: (1) a CDR1 sequence that has more than 80 % sequence identity with one of the CDR1 sequences mentioned in Table I; the CDR2 sequence listed in Table I that belongs to the same combination; and a CDR3 sequence mentioned in Table I that belongs to a different combination; or (2) a CDR1 sequence mentioned in Table I; a CDR2 sequence that has 3, 2 or 1 amino acid differences with the CDR2 sequence mentioned in Table I that belongs to the same combination; and more than 80% sequence identity with the CDR3 sequence listed in Table I that belongs to same different combination.

Particularly preferred Nanobodies of the invention may for example comprise a CDR1 sequence mentioned in Table I, a CDR2 sequence that has more than 80 % sequence identity with the CDR2 sequence mentioned in Table I that belongs to the same combination; and the CDR3 sequence mentioned in Table I that belongs to the same.

In the most preferred in the Nanobodies of the invention, the CDR1, CDR2 and CDR3 sequences present are suitably chosen from the one of the combinations of CDR1, CDR2 and CDR3 sequences, respectively, listed in Table I.

Preferably, when a CDR sequence is suitably chosen from the group of CDR sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the CDR sequences listed in Table I; and/or when a CDR sequence is suitably chosen from the group consisting of CDR sequences that have 3, 2 or only 1 amino acid difference(s) with one of the CDR sequences listed in Table I:

i) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the CDR sequence listed in Table I.

According to a non-limiting but preferred embodiment of the invention, the CDR sequences in the the Nanobodies of the invention are as defined above and are also such that the Nanobody of the invention binds to TNF-alpha with an dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter (M) or less, and preferably 10^{-7} to 10^{-12} moles/liter (M) or less and more preferably 10^{-8} to 10^{-12} moles/liter (M), and/or with an association constant (K_A)

of at least 10^7 M^{-1}, preferably at least 10^8 M^{-1}, more preferably at least 10^9 M^{-1}, such as at least 10^{12} M^{-1};and in particular with a K_D less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM. The K_D and K_A values of the Nanobody of the invention against TNF-alpha can be determined in a manner known per se, for example using the assay described herein.

According to another preferred, but non-limiting embodiment of the invention (a) CDR1 has a length of between 1 and 12 amino acid residues, and usually between 2 and 9 amino acid residues, such as 5, 6 or 7 amino acid residues; and/or (b) CDR2 has a length of between 13 and 24 amino acid residues, and usually between 15 and 21 amino acid residues, such as 16 and 17 amino acid residues; and/or (c) CDR3 has a length of between 2 and 35 amino acid residues, and usually between 3 and 30 amino acid residues, such as between 6 and 23 amino acid residues.

In one aspect, the invention provides Nanobodies against TNF-alpha that are better performing than Nanobody 3E, the best performing Nanobody according to WO 04/041862.

More specifically, some preferred aspects of this embodiment of the invention are:

XXI) A Nanobody against TNF-alpha, which consist of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), which has a Koff rate for TNF of better than 2.10-3 (1/s), preferably better than 1.10-3 (1/s); or a humanized variant of such a Nanobody.

XXII) A Nanobody against TNF-alpha, which consist of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than the EC50 value of Nanobody VHH 3E (SEQ ID NO:4) of WO 04/041862 in the same assay; or a humanized variant of such a Nanobody.

XXIII) A Nanobody against TNF-alpha, which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 12nM; or a humanized variant of such a Nanobody.

XXIV) A Nanobody against TNF-alpha, which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 5nM; or a humanized variant of such a Nanobody.

XXV) A Nanobody against TNF-alpha, which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 3nM; or a humanized variant of such a Nanobody;

with some particularly preferred aspects being:

- A Nanobody in accordance with any one of XXI) to XXV), which is a GLEW-class Nanobody.
- A Nanobody in accordance with any one of XXI) to XXV), which contains an arginine residue (R) at position 103.
- A Nanobody in accordance with any one of XXI) to XXV), which is a humanized Nanobody.
- A Nanobody in accordance with any one of XXI) to XXV), which contains a leucine residue (L) at position 108.
- A Nanobody in accordance with any one of XXI) to XXV), which has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the amino acid sequences of SEQ ID NO's 52 (TNF1), 76 (TNF13), 77 (TNF14), 95 (TNF29) or 96 (TNF30).
- A Nanobody in accordance with any one of XXI) to XXV), in which
  a) CDR1 comprises:
     - the amino acid sequence DYWMY; or
     - an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence DYWMY; or
     - an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence DYWMY;

  and

  b) CDR2 comprises:
     - the amino acid sequence EINTNGLITKYPDSVKG; or
     - an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence EINTNGLITKYPDSVKG; or
     - an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence EINTNGLITKYPDSVKG;

and

c) CDR3 comprises:

- the amino acid sequence SPSGFN; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence SPSGFN; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence SPSGFN.

- A Nanobody in accordance with any one of XXI) to XXV), in which CDR1 comprises the amino acid sequence DYWMY.
- A Nanobody in accordance with any one of XXI) to XXV), in which CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG.
- A Nanobody in accordance with any one of XXI) to XXV), in which CDR3 comprises the amino acid sequence SPSGFN
- A Nanobody in accordance with any one of XXI) to XXV), in which:
  - CDR1 comprises the amino acid sequence DYWMY; and CDR3 comprises the amino acid sequence SPSGFN; or
  - CDR1 comprises the amino acid sequence DYWMY; and CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG; or
  - CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG; and CDR3 comprises the amino acid sequence SPSGFN
- A Nanobody in accordance with any one of XXI) to XXV), in which CDR1 comprises the amino acid sequence DYWMY; and CDR3 comprises the amino acid sequence SPSGFN.
- A Nanobody in accordance with any one of XXI) to XXV), in which CDR1 comprises the amino acid sequence DYWMY; CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG and CDR3 comprises the amino acid sequence SPSGFN.
- A Nanobody in accordance with any one of XXI) to XXV), in which

a) CDR1 is:

- the amino acid sequence DYWMY; or

- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence DYWMY; or
- an amino acid sequences that has 2 or only 1 amino acid difference with the amino acid sequence DYWMY;

and in which:

b) CDR2 is:

- the amino acid sequence EINTNGLITKYPDSVKG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence EINTNGLITKYPDSVKG; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence EINTNGLITKYPDSVKG;

and in which

c) CDR3 is:

- the amino acid sequence SPSGFN; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence SPSGFN; or
- an amino acid sequences that has 2 or only 1 amino acid difference with the amino acid sequence SPSGFN.

- A Nanobody in accordance with any one of XXI) to XXV), in which CDR1 is the amino acid sequence DYWMY.
- A Nanobody in accordance with any one of XXI) to XXV), in which CDR2 is the amino acid sequence EINTNGLITKYPDSVKG.
- A Nanobody in accordance with any one of XXI) to XXV), in which CDR3 is the amino acid sequence SPSGFN
- A Nanobody in accordance with any one of XXI) to XXV), in which:
  - CDR1 is the amino acid sequence DYWMY; and CDR3 is the amino acid sequence SPSGFN; or
  - CDR1 is the amino acid sequence DYWMY; and CDR2 is the amino acid sequence EINTNGLITKYPDSVKG; or

- CDR2 is the amino acid sequence EINTNGLITKYPDSVKG; and CDR3 is the amino acid sequence SPSGFN

- A Nanobody in accordance with any one of XXI) to XXV), in which CDR1 is the amino acid sequence DYWMY; and CDR3 is the amino acid sequence SPSGFN.

- A Nanobody in accordance with any one of XXI) to XXV), in which CDR1 is the amino acid sequence DYWMY; CDR2 is the amino acid sequence EINTNGLITKYPDSVKG and CDR3 is the amino acid sequence SPSGFN.

- A Nanobody in accordance with any one of XXI) to XXV), in which
  - any amino acid substitution is preferably a conservative amino acid substitution; and/or
  - said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s).

and with some other particularly preferred aspects being:

- A Nanobody in accordance with any one of XXI) to XXV), which is a KERE-class Nanobody.

- A Nanobody in accordance with any one of XXI) to XXV), which has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the amino acid sequences of SEQ ID NO's 50 (TNF3), 83 (TNF20), 85 (TNF21), 85 (TNF22), 96 (TNF23) or 98 (TNF33).

- A Nanobody in accordance with any one of XXI) to XXV), in which

  a) CDR1 comprises:
     - the amino acid sequence NYYMG; or
     - an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence NYYMG; or
     - an amino acid sequences that has 2 or only 1 amino acid difference with the amino acid sequence NYYMG;

  and

  b) CDR2 comprises:
     - the amino acid sequence NISWRGYNIYYKDSVKG; or

- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence NISWRGYNIYYKDSVKG; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence NISWRGYNIYYKDSVKG;

and

c) CDR3 comprises:

- the amino acid sequence SILPLSDDPGWNTY; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence SILPLSDDPGWNTY; or
- an amino acid sequences that has 2 or only 1 amino acid difference with the amino acid sequence SILPLSDDPGWNTY.

- A Nanobody in accordance with any one of XXI) to XXV), in which CDR1 comprises the amino acid sequence NYYMG.
- A Nanobody in accordance with any one of XXI) to XXV), in which CDR2 comprises the amino acid sequence NISWRGYNIYYKDSVKG.
- A Nanobody in accordance with any one of XXI) to XXV), in which CDR3 comprises the amino acid sequence SILPLSDDPGWNTY.
- A Nanobody in accordance with any one of XXI) to XXV), in which:
  - CDR1 comprises the amino acid sequence NYYMG; and CDR3 comprises the amino acid sequence SILPLSDDPGWNTY; or
  - CDR1 comprises the amino acid sequence NYYMG; and CDR2 comprises the amino acid sequence NISWRGYNIYYKDSVKG; or
  - CDR2 comprises the amino acid sequence NISWRGYNIYYKDSVKG; and CDR3 comprises the amino acid sequence SILPLSDDPGWNTY.
- A Nanobody in accordance with any one of XXI) to XXV), in which CDR1 comprises the amino acid sequence NYYMG; CDR2 comprises the amino acid sequence SILPLSDDPGWNTY and CDR3 comprises the amino acid sequence ILPLSDDPGWNTY.
- A Nanobody in accordance with any one of XXI) to XXV), in which

64

a) CDR1 is:

- the amino acid sequence NYYMG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence NYYMG; or
- an amino acid sequences that has 2 or only 1 amino acid difference with the amino acid sequence NYYMG;

and

b) CDR2 is:

- the amino acid sequence NISWRGYNIYYKDSVKG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence NISWRGYNIYYKDSVKG; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence NISWRGYNIYYKDSVKG;

and

c) CDR3 is:

- the amino acid sequence SILPLSDDPGWNTY; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence SILPLSDDPGWNTY; or
- an amino acid sequences that has 2 or only 1 amino acid difference with the amino acid sequence SILPLSDDPGWNTY.

- A Nanobody in accordance with any one of XXI) to XXV), in which CDR1 is the amino acid sequence NYYMG.
- A Nanobody in accordance with any one of XXI) to XXV), in which CDR2 is the amino acid sequence NISWRGYNIYYKDSVKG.
- A Nanobody in accordance with any one of XXI) to XXV), in which CDR3 is the amino acid sequence SILPLSDDPGWNTY.
- A Nanobody in accordance with any one of XXI) to XXV), in which:

- CDR1 is the amino acid sequence NYYMG; and CDR3 is the amino acid sequence SILPLSDDPGWNTY; or
- CDR1 is the amino acid sequence NYYMG; and CDR2 is the amino acid sequence NISWRGYNIYYKDSVKG; or
- CDR2 is the amino acid sequence NISWRGYNIYYKDSVKG; and CDR3 is the amino acid sequence SILPLSDDPGWNTY.

- A Nanobody in accordance with any one of XXI) to XXV), in which CDR1 is the amino acid sequence NYYMG; CDR2 is the amino acid sequence SILPLSDDPGWNTY and CDR3 is the amino acid sequence ILPLSDDPGWNTY.
- A Nanobody in accordance with any one of XXI) to XXV), in which
  - any amino acid substitution is preferably a conservative amino acid substitution; and/or
  - said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s).
- A Nanobody in accordance with any one of XXI) to XXV), which is a humanized Nanobody.

and with yet some other particularly preferred aspects being:

XXVI) A protein or polypeptide, which comprises or essentially consists of at least one Nanobody in accordance with any one of XXI) to XXV).

XXVII) A protein or polypeptide, which comprises two Nanobodies in accordance with any one of XXI) to XXV).

XXVIII) A protein or polypeptide, which comprises two Nanobodies in accordance with any one of XXI) to XXV), and which is such that said protein or polypeptide, upon binding to a TNF trimer, is capable inhibiting or reducing the TNF receptor crosslinking that is mediated by said TNF trimer and/or the signal transduction that is mediated by such receptor crosslinking..

XXIX) A protein or polypeptide, which comprises two Nanobodies in accordance with any one of XXI) to XXV), and which is capable of intramolecular binding to at least two TNF receptor binding sites on a TNF trimer.

XXX) A protein or polypeptide, which comprises two Nanobodies in accordance with any one of XXI) to XXV), linked via a suitable linker.

66

XXXI) A protein or polypeptide, which comprises two Nanobodies in accordance with any one of XXI) to XXV), linked via a suitable linker, and which is pegylated.

XXXII) A protein or polypeptide which comprises two Nanobodies in accordance with any one of XXI) to XXV), and which further comprises at least one Nanobody directed against human serum albumin.

XXXIII) A protein or polypeptide which comprises two Nanobodies in accordance with any one of XXI) to XXV), and which further comprises at least one Nanobody directed against human serum albumin, and which is such that said protein or polypeptide, upon binding to a TNF trimer, is capable inhibiting or reducing the TNF receptor crosslinking that is mediated by said TNF trimer and/or the signal transduction that is mediated by such receptor crosslinking..

XXXIV) A protein or polypeptide which comprises two Nanobodies in accordance with any one of XXI) to XXV), and which further comprises at least one Nanobody directed against human serum albumin and which is capable of intramolecular binding to at least two TNF receptor binding sites on a TNF trimer.

XXXV) A protein or polypeptide which comprises two Nanobodies in accordance with any one of XXI) to XXV), and which further comprises one Nanobody directed against human serum albumin, in which each of the two Nanobodies in accordance with any one of XXI) to XXV) is linked, optionally via a suitable linker, to the one Nanobody directed against human serum albumin.

XXXVI) A protein or polypeptide which comprises two Nanobodies in accordance with any one of XXI) to XXV), and which further comprises one Nanobody directed against human serum albumin, in which each of the two Nanobodies in accordance with any one of XXI) to XXV) is linked, optionally via a suitable linker, to the one Nanobody directed against human serum albumin, and which is such that said protein or polypeptide, upon binding to a TNF trimer, is capable inhibiting or reducing the TNF receptor crosslinking that is mediated by said TNF trimer and/or the signal transduction that is mediated by such receptor crosslinking..

XXXVII) A protein or polypeptide which comprises two Nanobodies in accordance with any one of XXI) to XXV), and which further comprises one Nanobody directed against human serum albumin, in which each of the two Nanobodies in accordance with any one of XXI) to XXV) is linked, optionally via a suitable linker, to the one

Nanobody directed against human serum albumin, and which is capable of intramolecular binding to at least two TNF receptor binding sites on a TNF trimer.

- A protein or polypeptide in accordance with any one of XXVI) to XXXVII), in which the at least one Nanobody directed against human serum albumin is a humanized Nanobody.
- A protein or polypeptide in accordance with any one of XXVI) to XXXVII), in which the at least one Nanobody directed against human serum albumin is a humanized variant of the Nanobody ALB 1 (SEQ ID NO: 63).
- A protein or polypeptide in accordance with any one of XXVI) to XXXVII), in which the at least one Nanobody directed against human serum albumin is a chosen from the group consisting of ALB 3 (SEQ ID NO: 87), ALB 4 (SEQ ID NO: 88), ALB 5 (SEQ ID NO: 89), ALB 6 (SEQ ID NO: 100), ALB 7 (SEQ ID NO: 101), ALB 8 (SEQ ID NO: 102) ALB 9 (SEQ ID NO: 103) and ALB 10 (SEQ ID NO: 104).
- A protein or polypeptide in accordance with any one of XXVI) to XXXVII), in which the at least one Nanobody directed against human serum albumin is ALB 8.
- A protein or polypeptide in accordance with any one of XXVI) to XXXVII), which comprises or essentially consists of two humanized Nanobodies Nanobodies in accordance with any one of XXI) to XXV), and one humanized variant of the Nanobody ALB 1 (SEQ ID NO: 63).

It should be noted that when a Nanobody is mentioned above as being "*in accordance with any one of XXI) to XXV) above*", it is at least according to one of XXI) to XXV), may be according to two or more of XXI) to XXV), and may also include any one or more of the other aspects that are indicated as being "in accordance with any one of XXI) to XXV) above. Similarly, when a protein or polypeptide is mentioned above as being "*in accordance with any one of XXVI) to XXXVII) above*", it is at least according to one of XXVI) to XXXVII), may be according to two or more of XXVI) to XXXVII), and may also include any one or more of the other aspects that are indicated as being "in accordance with any one of XXVI) to XXXVII) above.

A clone that has been found to be particularly useful as an anti-TNF Nanobody is the clone PMP1C2 (TNF1). As can be seen from the comparative data from the KYM-assay in Table 39, TNF1 has an EC_{50} value that is more then 4 times better than the best monovalent Nanobody described in WO 04/41862 (Nanobody 3E), i.e. 2,466 nM for

PMP1C2 vs. 12 nM for 3E (As can be seen from Table 39, all Nanobodies TNF1 to TNF 9 of the invention gave a better EC50 value in this assay than 3E). In this respect, it should also be noted that Nanobody 3E from WO 04/41862 belongs to the "KERE class" (as described herein), and can therefore be humanized to a lesser degree than Nanobody PMP1C2 (which belongs to the "GLEW class"). When Nanobody PMP1C2 is compared to the Nanobody 1A from WO 04/41862, a GLEW-class Nanobody with the highest degree in sequence homology with PMP1C2 (in both the CDR's and the frameworks), the EC_{50} value obtained for PMP1C2 in the KYM assay is more than 50 times better, i.e. 2.466 nM for PMP1C2 compared to 100 nM for 1A.

Accordingly, Nanobodies that comprise one or more, preferably any two and more preferably all three of the CDR's present in the clone PMP1C2 (or CDR sequences that are derived therefrom or correspond thereto) are particularly preferred in the invention. Also, these Nanobodies preferably belong to the "103 P,R,S group" (as defined herein), and most preferably have an R at position 103, and preferably also have GLEW or a GLEW-like sequence at positions 44-47. Also, when these Nanobodies belong to the "103 P, R, S group" (and in particular when they have an R at position 103), one preferred, but non-limiting humanizing substitution is 108Q to 108L. Other preferred, but non-limiting humanizing substitutions in these preferred Nanobodies are one or more of those present in the humanized variants of TNF1 described herein, such as TNF13, TNF14, TNF 29 or TNF30, as will immediately be clear from a comparison between the sequence of TNF1 and these humanized sequences.

Thus, in a particularly preferred Nanobody of the invention, at least one of the CDR1, CDR2 and CDR3 sequences present is suitably chosen from the group consisting of the CDR1 sequence of SEQ ID NO: 164, the CDR2 sequence of SEQ ID NO: 232, and the CDR3 sequence of SEQ ID NO: 300, respectively (i.e. the CDR sequences present in clone TNF1), or from the group of CDR1, CDR2 and CDR3 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% "sequence identity" (as defined herein) with the CDR1 sequence of SEQ ID NO: 164, the CDR2 sequence of SEQ ID NO: 232, and the CDR3 sequence of SEQ ID NO: 300, respectively; and/or from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with the CDR1 sequence of SEQ ID NO: 164, the CDR2 sequence of SEQ ID NO: 232, and the CDR3 sequence of SEQ ID NO: 300, respectively.

Preferably, in these preferred Nanobodies of the invention, at least two of the CDR1, CDR2 and CDR3 sequences present are suitably chosen from the group consisting of the CDR1 sequence of SEQ ID NO: 164, the CDR2 sequence of SEQ ID NO: 232, and the CDR3 sequence of SEQ ID NO: 300, respectively (i.e. the CDR sequences present in clone TNF1), or from the group consisting of CDR1, CDR2 and CDR3 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the CDR1 sequence of SEQ ID NO: 164, the CDR2 sequence of SEQ ID NO: 232, and the CDR3 sequence of SEQ ID NO: 300, respectively; and/or from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, that have 3, 2 or only 1 "amino acid difference(s)" with the CDR1 sequence of SEQ ID NO: 164, the CDR2 sequence of SEQ ID NO: 232, and the CDR3 sequence of SEQ ID NO: 300, respectively.

Most preferably, in these preferred Nanobodies of the invention, all three CDR1, CDR2 and CDR3 sequences present are suitably chosen from the group consisting of the CDR1 sequence of SEQ ID NO: 164, the CDR2 sequence of SEQ ID NO: 232, and the CDR3 sequence of SEQ ID NO: 300, respectively (i.e. the CDR sequences present in clone TNF1), or from the group of CDR1, CDR2 and CDR3 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the CDR1 sequence of SEQ ID NO: 164, the CDR2 sequence of SEQ ID NO: 232, and the CDR3 sequence of SEQ ID NO: 300, respectively; and/or from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, that have 3, 2 or only 1 amino acid difference(s) with the CDR1 sequence of SEQ ID NO: 164, the CDR2 sequence of SEQ ID NO: 232, and the CDR3 sequence of SEQ ID NO: 300, respectively.

Even more preferably, in these preferred Nanobodies of the invention, at least one of the CDR1, CDR2 and CDR3 sequences present is suitably chosen from the group consisting of the CDR1 sequence of SEQ ID NO: 164, the CDR2 sequence of SEQ ID NO: 232, and the CDR3 sequence of SEQ ID NO: 300, respectively (i.e. the CDR sequences present in clone TNF1). Preferably, in this embodiment, at least one or preferably both of the other two CDR sequences present are suitably suitably chosen from CDR sequences that that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the CDR1 sequence of SEQ ID NO: 164, the CDR2 sequence of SEQ ID NO: 232, and the CDR3 sequence of

SEQ ID NO: 300, respectively; and/or suitably chosen from the group consisting of the CDR sequences that have 3, 2 or only 1 amino acid difference(s) with the CDR1 sequence of SEQ ID NO: 164, the CDR2 sequence of SEQ ID NO: 232, and the CDR3 sequence of SEQ ID NO: 300, respectively.

Even more preferably, in these preferred Nanobodies of the invention, at least two of the CDR1, CDR2 and CDR3 sequences present are suitably chosen from the group consisting of the CDR1 sequence of SEQ ID NO: 164, the CDR2 sequence of SEQ ID NO: 232, and the CDR3 sequence of SEQ ID NO: 300, respectively (i.e. the CDR sequences present in clone TNF1). Preferably, in this embodiment, the remaining CDR sequence present are suitably chosen from the group of CDR sequences that that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the CDR1 sequence of SEQ ID NO: 164, the CDR2 sequence of SEQ ID NO: 232, and the CDR3 sequence of SEQ ID NO: 300, respectively; and/or from the group consisting of CDR sequences that have 3, 2 or only 1 amino acid difference(s) with the CDR1 sequence of SEQ ID NO: 164, the CDR2 sequence of SEQ ID NO: 232, and the CDR3 sequence of SEQ ID NO: 300, respectively.

Particularly preferred Nanobodies of the invention comprise the CDR1 sequence of SEQ ID NO: 164, the CDR2 sequence of SEQ ID NO: 232, and the CDR3 sequence of SEQ ID NO: 300, respectively (i.e. the CDR sequences present in clone TNF1).

Nanobodies with the above CDR sequences preferably have framework sequences that are as further defined herein. Some particularly preferred, but non-limiting combinations of framework sequences can be seen in the above Table I. As will be clear to the skilled person, a combination of FR1, FR2, FR3 and FR4 sequences that occur in the same clone (i.e. FR1, FR2, FR3 and FR4 sequences which are mentioned on the same line in Table I) will usually be preferred (although the invention in its broadest sense is not limited thereto, and also comprises other suitable combinations of the framework sequences mentioned in Table I).

More specifically, some preferred aspects of this embodiment of the invention are:

XXXVIII) A nanobody against TNF-alpha, which consist of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), in which:

a) CDR1 comprises:

- the amino acid sequence DYWMY; or

- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence DYWMY; or
- an amino acid sequences that has only 1 amino acid difference with the amino acid sequence DYWMY;

and

b) CDR2 comprises:

- the amino acid sequence EINTNGLITKYPDSVKG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence EINTNGLITKYPDSVKG; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence EINTNGLITKYPDSVKG;

and

c) CDR3 comprises:

- the amino acid sequence SPSGFN; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence SPSGFN; or
- an amino acid sequences that has only 1 amino acid difference with the amino acid sequence SPSGFN.

- A nanobody in accordance with XXXVIII) , in which CDR1 comprises the amino acid sequence DYWMY.
- A nanobody in accordance with XXXVIII) , in which CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG.
- A nanobody in accordance with XXXVIII) , in which CDR3 comprises the amino acid sequence SPSGFN.
- A nanobody in accordance with XXXVIII) , in which:
  - CDR1 comprises the amino acid sequence DYWMY; and CDR3 comprises the amino acid sequence SPSGFN; or
  - CDR1 comprises the amino acid sequence DYWMY; and CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG; or

- CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG; and CDR3 comprises the amino acid sequence SPSGFN

- A nanobody in accordance with XXXVIII) , in which CDR1 comprises the amino acid sequence DYWMY; and CDR3 comprises the amino acid sequence SPSGFN.
- A nanobody in accordance with XXXVIII) in which CDR1 comprises the amino acid sequence DYWMY; CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG and CDR3 comprises the amino acid sequence SPSGFN.
- A Nanobody in accordance with XXXVIII) , in which
  - any amino acid substitution is preferably a conservative amino acid substitution; and/or
  - said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s).
- A Nanobody in accordance with XXXVIII) , which is a GLEW-class Nanobody.
- A Nanobody in accordance with XXXVIII) , which contains an arginine residue (R) at position 103.
- A Nanobody in accordance with XXXVIII) , which has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the amino acid sequences of SEQ ID NO's 52 (TNF1), 76 (TNF13), 77 (TNF14), 95 (TNF29) or 96 (TNF30).
- A Nanobody in accordance with XXXVIII) , which is a humanized Nanobody.
- A Nanobody in accordance with XXXVIII) , which contains a leucine residue (L) at position 108.
- A Nanobody in accordance with XXXVIII) , which has a K_{off} rate for TNF of better than 2.10-3 (1/s), preferably better than 1.10-3 (1/s); or a humanized variant of such a Nanobody;
- A Nanobody in accordance with XXXVIII) , which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than the EC50 value of Nanobody VHH 3E (SEQ ID NO:4) of WO 04/041862 in the same assay; or a humanized variant of such a Nanobody.
- A Nanobody in accordance with XXXVIII) , which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 5nM; or a humanized variant of such a Nanobody.

- A Nanobody in accordance with XXXVIII) , which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 3nM; or a humanized variant of such a Nanobody.

XXXIX) A Nanobody against TNF-alpha, which consist of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), in which

a) CDR1 is:

- the amino acid sequence DYWMY; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence DYWMY; or
- an amino acid sequences that has only 1 amino acid difference with the amino acid sequence DYWMY;

and in which:

b) CDR2 is:

- the amino acid sequence EINTNGLITKYPDSVKG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence EINTNGLITKYPDSVKG; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence EINTNGLITKYPDSVKG;

and in which

c) CDR3 is:

- the amino acid sequence SPSGFN; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence SPSGFN; or
- an amino acid sequences that has only 1 amino acid difference with the amino acid sequence SPSGFN.

- A Nanobody in accordance with XXXIX) , in which CDR1 is the amino acid sequence DYWMY.

- A Nanobody in accordance with XXXIX) , in which CDR2 is the amino acid sequence EINTNGLITKYPDSVKG.
- A Nanobody in accordance with XXXIX) , in which CDR3 is the amino acid sequence SPSGFN
- A Nanobody in accordance with XXXIX) , in which:
  - CDR1 is the amino acid sequence DYWMY; and CDR3 is the amino acid sequence SPSGFN; or
  - CDR1 is the amino acid sequence DYWMY; and CDR2 is the amino acid sequence EINTNGLITKYPDSVKG; or
  - CDR2 is the amino acid sequence EINTNGLITKYPDSVKG; and CDR3 is the amino acid sequence SPSGFN
- A Nanobody in accordance with XXXIX) , in which CDR1 is the amino acid sequence DYWMY; and CDR3 is the amino acid sequence SPSGFN.
- A Nanobody in accordance with XXXIX) , in which CDR1 is the amino acid sequence DYWMY; CDR2 is the amino acid sequence EINTNGLITKYPDSVKG and CDR3 is the amino acid sequence SPSGFN.
- A Nanobody in accordance with XXXIX) , in which:
  - any amino acid substitution is preferably a conservative amino acid substitution; and/or
  - said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s).
- A Nanobody in accordance with XXXIX) , which is a GLEW-class Nanobody.
- A Nanobody in accordance with XXXIX) , which contains an arginine residue (R) at position 103.
- A Nanobody in accordance with XXXIX) , which has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the amino acid sequences of SEQ ID NO's 52 (TNF1), 76 (TNF13), 77 (TNF14), 95 (TNF29) or 96 (TNF30).
- A Nanobody in accordance with XXXIX) , which is a humanized Nanobody.
- A Nanobody in accordance with XXXIX) , which contains a leucine residue (L) at position 108.

- A Nanobody in accordance with XXXIX) , which has a K_{off} rate for TNF of better than 2.10-3 (1/s), preferably better than 1.10-3 (1/s); or a humanized variant of such a Nanobody.
- A Nanobody in accordance with XXXIX) , which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than the EC50 value of Nanobody VHH 3E (SEQ ID NO:4) of WO 04/041862 in the same assay or a humanized variant of such a Nanobody.
- A Nanobody in accordance with XXXIX) , which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 5nM; or a humanized variant of such a Nanobody.
- A Nanobody in accordance with XXXIX) , which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 3nM; or a humanized variant of such a Nanobody.
- A Nanobody in accordance with XXXIX) , which is chosen from the group consisting of TNF 13 (SEQ ID NO: 76), TNF 14 (SEQ ID NO: 77), TNF 29 (SEQ ID NO: 95) and TNF 30 (SEQ ID NO:96).
- A Nanobody in accordance with XXXIX) , which is TNF 30 (SEQ ID NO: 96);

with some other preferred aspects being:

XL) A protein or polypeptide, which comprises or essentially consists of a Nanobody in accordance with XXXVIII) or XXXIX).

XLI) A protein or polypeptide, which comprises or essentially consists of at least one Nanobody in accordance with XXXVIII) or XXXIX) .

- A protein or polypeptide in accordance with any one of XL) or XLI), which comprises two Nanobodies in accordance with XXXVIII) or XXXIX) .
- A protein or polypeptide in accordance with any one of XL) or XLI),, which comprises two Nanobodies in accordance with XXXVIII) or XXXIX) , and which is such that said protein or polypeptide, upon binding to a TNF trimer, is capable inhibiting or reducing the TNF receptor crosslinking that is mediated by said TNF trimer and/or the signal transduction that is mediated by such receptor crosslinking..
- A protein or polypeptide in accordance with any one of XL) or XLI),, which comprises two Nanobodies in accordance with XXXVIII) or XXXIX) , and which is capable of intramolecular binding to at least two TNF receptor binding sites on a TNF trimer.

- A protein or polypeptide in accordance with any one of XL) or XLI), which comprises two Nanobodies in accordance with XXXVIII) or XXXIX) , which are directly linked to each other or linked to each other via a linker.
- A protein or polypeptide in accordance with any one of XL) or XLI), which comprises two Nanobodies in accordance with XXXVIII) or XXXIX), which are linked to each other via an amino acid sequence.
- A protein or polypeptide in accordance with any one of XL) or XLI), which comprises two Nanobodies in accordance with XXXVIII) or XXXIX), which are linked to each other via an amino acid sequence (such as, without limitation, an amino acid sequence that comprises glycine and serine residues) that comprises at least 14 amino acids, more preferably at least 17 amino acids, such as about 20-40 amino acids (such as the linker GS30).
- A protein or polypeptide in accordance with any one of XL) or XLI), which comprises or essentially consists of the polypeptide TNF 7 (SEQ ID NO: 73), in which both Nanobodies TNF 1 have been humanized.
- A protein or polypeptide in accordance with any one of XL) or XLI), which comprises or essentially consists the polypeptide TNF 55 (SEQ ID NO: 419) or TNF 56 (SEQ ID NO: 420).
- A protein or polypeptide in accordance with any one of XL) or XLI), which is pegylated.
- A protein or polypeptide in accordance with any one of XL) or XLI),which comprises two Nanobodies in accordance with XXXVIII) or XXXIX) , and which is such that said protein or polypeptide, upon binding to a TNF trimer, is capable inhibiting or reducing the TNF receptor crosslinking that is mediated by said TNF trimer and/or the signal transduction that is mediated by such receptor crosslinking; and/or which is such that said protein or polypeptide is capable of intramolecular binding to at least two TNF receptor binding sites on a TNF trimer, and which protein or polypeptide further comprises at least one Nanobody directed against human serum albumin.
- A protein or polypeptide in accordance with any one of XL) or XLI),, which comprises two Nanobodies in accordance with XXXVIII) or XXXIX), and which protein or polypeptide further comprises at least one Nanobody directed against human serum albumin, in which the two Nanobodies in accordance with XXXVIII)

or XXXIX) are linked to each other via the at least one Nanobody directed against human serum albumin, and in which the two Nanobodies in accordance with XXXVIII) or XXXIX) are either linked directly to the at least one Nanobody directed against human serum albumin, or are linked to the at least one Nanobody directed against human serum albumin via a linker.

- A protein or polypeptide in accordance with any one of XL) or XLI),, which comprises two Nanobodies in accordance with XXXVIII) or XXXIX), and which protein or polypeptide further comprises at least one Nanobody directed against human serum albumin, in which the two Nanobodies in accordance with XXXVIII) or XXXIX) are linked to each other via the at least one Nanobody directed against human serum albumin, and in which the two Nanobodies in accordance with XXXVIII) or XXXIX) are either linked directly to the at least one Nanobody directed against human serum albumin, or are linked to the at least one Nanobody directed against human serum albumin via a linker, in which the linker is an amino acid sequence (such as, without limitation, a linker that comprises glycine and serine residues), and in particular an amino acid sequence that comprises between 3 and 40 amino acid residues, such as between 5 and 15 amino acid residues (such as the linker GS9).

- A protein or polypeptide in accordance with any one of XL) or XLI), which comprises two Nanobodies in accordance with XXXVIII) or XXXIX), and which protein or polypeptide further comprises at least one Nanobody directed against human serum albumin, in which the two Nanobodies in accordance with XXXVIII) or XXXIX) are linked to each other via the at least one Nanobody directed against human serum albumin, and in which the two Nanobodies in accordance with XXXVIII) or XXXIX) are either linked directly to the at least one Nanobody directed against human serum albumin, or are linked to the at least one Nanobody directed against human serum albumin via a linker, and which protein or polypeptide is such that said protein or polypeptide, upon binding to a TNF trimer, is capable inhibiting or reducing the TNF receptor crosslinking that is mediated by said TNF trimer and/or the signal transduction that is mediated by such receptor crosslinking; and/or which protein or polypeptide is such that said protein or polypeptide is capable of intramolecular binding to at least two TNF receptor binding sites on a TNF trimer.

- A protein or polypeptide in accordance with any one of XL) or XLI), in which the at least one Nanobody directed against human serum albumin is a humanized Nanobody.
- A protein or polypeptide in accordance with any one of XL) or XLI), in which the at least one Nanobody directed against human serum albumin is a humanized variant of the Nanobody ALB 1 (SEQ ID NO: 63).
- A protein or polypeptide in accordance with any one of XL) or XLI), in which the at least one Nanobody directed against human serum albumin is a chosen from the group consisting of ALB 3 (SEQ ID NO: 87), ALB 4 (SEQ ID NO: 88), ALB 5 (SEQ ID NO: 89), ALB 6 (SEQ ID NO: 100), ALB 7 (SEQ ID NO: 101), ALB 8 (SEQ ID NO: 102) ALB 9 (SEQ ID NO: 103) and ALB 10 (SEQ ID NO: 104).
- A protein or polypeptide in accordance with any one of XL) or XLI), in which the at least one Nanobody directed against human serum albumin is ALB 8.
- A protein or polypeptide in accordance with any one of XL) or XLI), which comprises or essentially consists of two humanized Nanobodies in accordance with any one of XL) or XLI) and one humanized variant of the Nanobody ALB 1 (SEQ ID NO: 63).
- A protein or polypeptide in accordance with any one of XL) or XLI), which comprises or essentially consists of the polypeptide TNF24 (SEQ ID NO: 90), in which both the Nanobody TNF 1 as well as the Nanobody ALB 1 has been humanized.
- A protein or polypeptide in accordance with any one of XL) or XLI), which comprises or essentially consists of two Nanobodies TNF 30 and one Nanobody ALB 8.
- A protein or polypeptide in accordance with any one of XL) or XLI), which comprises or essentially consists the polypeptide TNF 60 (SEQ ID NO: 417).

It should be noted that when a Nanobody is mentioned above as being "*in accordance with XXXVIII" or "in accordance with XXXIX*", it is at least according to one of XXXVIII) and/or XXXIX), and may also include any one or more of the other aspects that are indicated as being "in accordance with XXXVIII" or "in accordance with XXXIX" above. Similarly, when a protein or polypeptide is mentioned above as being "*in accordance with any one of XL) or XLI)*", it is at least according to one of XL) to XLI), may be according to two or more of VI) to XVIII), and may also include any one or more

of the other aspects that are indicated as being "in accordance with any one of XL) or XLI) above.

For the Nanobodies based on Nanobody TNF1 above (including but not limited to the humanized Nanobodies), the framework sequences may generally be as described herein, and preferably are as follows:

a) FR1 comprises or is:

- the amino acid sequence of SEQ ID NO: 130;or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 130; or
- an amino acid sequences that has only 1 amino acid difference with the amino acid sequence of SEQ ID NO: 130;

and

b) FR2 comprises or is:

- the amino acid sequence of SEQ ID NO: 198; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 198; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence of SEQ ID NO: 198;

and

c) FR3 comprises or is:

- the amino acid sequence of SEQ ID NO: 266; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 266; or
- an amino acid sequences that has only 1 amino acid difference with the amino acid sequence of SEQ ID NO: 266.

and

d) FR4 comprises or is:

- the amino acid sequence of SEQ ID NO: 334; or

- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 334; or
- an amino acid sequences that has only 1 amino acid difference with the amino acid sequence of SEQ ID NO: 334;

in which the amino acid differences present in the framework sequences are more preferably as described herein.

Nanobodies against TNF-alpha, which have framework regions as described above (i.e. similar to TNF1), and in which at least one of the framework regions (such as any two, any three or all four framework regions) have been humanized, form a further aspect of the invention. Such Nanobodies may in particular have CDR's that are such that the Nanobody has a Koff rate for TNF of better than 2.10-3 (1/s), preferably better than 1.10-3 (1/s); and/or have CDR's that are such that the Nanobody has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than the EC50 value of Nanobody VHH 3E (SEQ ID NO:4) of WO 04/041862 in the same assay; and in particular better than 12nM, more in particular better than 5 nM, even more in particular better than 3nM. Also, or alternatively, such Nanobodies are preferably directed against the same epitope of TNF (i.e. the TNF trimer) as TNF1.

In particular, the invention relates to a Nanoobody against TNF-alpha, which is a humanized variant of a Nanobody against TNF-alpha, which Nanobody against TNF-alpha has the following framework sequences: FR1: SEQ ID NO: 130; FR2: SEQ ID NO: 198; FR3: SEQ ID NO: 266; and FR4: SEQ ID NO: 334. Such a Nanobody may in particular have CDR's that are such that the Nanobody has a Koff rate for TNF of better than 2.10-3 (1/s), preferably better than 1.10-3 (1/s); and/or have CDR's that are such that the Nanobody has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than the EC50 value of Nanobody VHH 3E (SEQ ID NO:4) of WO 04/041862 in the same assay; and in particular better than 12nM, more in particular better than 5 nM, even more in particular better than 3nM. Also, or alternatively, such Nanobodies are preferably directed against the same epitope of TNF (i.e. the TNF trimer) as TNF1.

Another clone that has been found to be particularly useful as an anti-TNF Nanobody is the clone PMP5F10 (TNF3, SEQ ID NO: 60). As can be seen from the

comparative data from the KYM-assay in Table 39, TNF3 has an EC_{50} value that is more than 15 times better than the best monovalent Nanobody described in WO 04/41862.

Accordingly, Nanobodies that comprise one or more, preferably any two and more preferably all three of the CDR's present in the clone PMP5F10 (or CDR sequences that are derived therefrom or correspond thereto) are particularly preferred in the invention. Also, these Nanobodies preferably belong to the KERE class.

More specifically, some preferred aspects of this embodiment of the invention are:

XLII) A Nanobody against TNF-alpha, which consist of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), in which

a) CDR1 comprises:

- the amino acid sequence NYYMG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence NYYMG; or
- an amino acid sequences that has 2 or only 1 amino acid difference with the amino acid sequence NYYMG;

and

b) CDR2 comprises:

- the amino acid sequence NISWRGYNIYYKDSVKG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence NISWRGYNIYYKDSVKG; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence NISWRGYNIYYKDSVKG;

and

c) CDR3 comprises:

- the amino acid sequence SILPLSDDPGWNTY; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence SILPLSDDPGWNTY; or

- an amino acid sequences that has 2 or only 1 amino acid difference with the amino acid sequence SILPLSDDPGWNTY.

- A Nanobody in accordance with XLII), in which CDR1 comprises the amino acid sequence NYYMG.
- A Nanobody in accordance with XLII), in which CDR2 comprises the amino acid sequence NISWRGYNIYYKDSVKG.
- A Nanobody in accordance with XLII), in which CDR3 comprises the amino acid sequence SILPLSDDPGWNTY.
- A Nanobody in accordance with XLII), in which:
  - CDR1 comprises the amino acid sequence NYYMG; and CDR3 comprises the amino acid sequence SILPLSDDPGWNTY; or
  - CDR1 comprises the amino acid sequence NYYMG; and CDR2 comprises the amino acid sequence NISWRGYNIYYKDSVKG; or
  - CDR2 comprises the amino acid sequence NISWRGYNIYYKDSVKG; and CDR3 comprises the amino acid sequence SILPLSDDPGWNTY.
- A Nanobody in accordance with XLII), in which CDR1 comprises the amino acid sequence NYYMG; CDR2 comprises the amino acid sequence SILPLSDDPGWNTY and CDR3 comprises the amino acid sequence ILPLSDDPGWNTY.
- A Nanobody in accordance with XLII), in which
  - any amino acid substitution is preferably a conservative amino acid substitution; and/or
  - said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s).
- A Nanobody in accordance with XLII), which is a KERE-class Nanobody.
- A Nanobody in accordance with XLII), which has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the amino acid sequences of SEQ ID NO's 50 (TNF3), 83 (TNF20), 85 (TNF21), 85 (TNF22), 96 (TNF23) or 99 (TNF33).
- A Nanobody in accordance with XLII), which is a humanized Nanobody.

- A Nanobody in accordance with XLII), which has a K_{off} rate for TNF of better than 2.10^{-3} (1/s), preferably better than 1.10^{-3} (1/s) ; or a humanized variant of such a Nanobody.
- A Nanobody in accordance with XLII), which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than the EC50 value of Nanobody VHH 3E (SEQ ID NO:4) of WO 04/041862 in the same assay; or a humanized variant of such a Nanobody.
- A Nanobody in accordance with XLII), which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 5nM; or a humanized variant of such a Nanobody.
- A Nanobody in accordance with XLII), which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 3nM; or a humanized variant of such a Nanobody.

XLIII) A Nanobody against TNF-alpha, which consist of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), in which

a) CDR1 is:

- the amino acid sequence NYYMG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence NYYMG; or
- an amino acid sequences that has 2 or only 1 amino acid difference with the amino acid sequence NYYMG;

and

b) CDR2 is:

- the amino acid sequence NISWRGYNIYYKDSVKG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence NISWRGYNIYYKDSVKG; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence NISWRGYNIYYKDSVKG;

and

c) CDR3 is:

- the amino acid sequence SILPLSDDPGWNTY; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence SILPLSDDPGWNTY; or
- an amino acid sequences that has 2 or only 1 amino acid difference with the amino acid sequence SILPLSDDPGWNTY.

- A Nanobody in accordance with XLIII), in which CDR1 is the amino acid sequence NYYMG.
- A Nanobody in accordance with XLIII), in which CDR2 is the amino acid sequence NISWRGYNIYYKDSVKG.
- A Nanobody in accordance with XLIII), in which CDR3 is the amino acid sequence SILPLSDDPGWNTY.
- A Nanobody in accordance with XLIII), in which:
  - CDR1 is the amino acid sequence NYYMG; and CDR3 is the amino acid sequence SILPLSDDPGWNTY; or
  - CDR1 is the amino acid sequence NYYMG; and CDR2 is the amino acid sequence NISWRGYNIYYKDSVKG; or
  - CDR2 is the amino acid sequence NISWRGYNIYYKDSVKG; and CDR3 is the amino acid sequence SILPLSDDPGWNTY.
- A Nanobody in accordance with XLIII), in which CDR1 is the amino acid sequence NYYMG; CDR2 is the amino acid sequence SILPLSDDPGWNTY and CDR3 is the amino acid sequence ILPLSDDPGWNTY.
- A Nanobody in accordance with XLIII), in which
  - any amino acid substitution is preferably a conservative amino acid substitution; and/or
  - said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s).
- A Nanobody in accordance with XLIII), which is a KERE-class Nanobody.
- A Nanobody in accordance with XLIII), which has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence

identity (as defined herein) with one of the amino acid sequences of SEQ ID NO's 50 (TNF3), 83 (TNF20), 85 (TNF21), 85 (TNF22), 96 (TNF23) or 99 (TNF33).

- A Nanobody in accordance with XLIII), which is a humanized Nanobody.
- A Nanobody in accordance with XLIII), which has a K_{off} rate for TNF of better than 2.10^{-3} (1/s), preferably better than 2.10^{-3} (1/s) ; or a humanized variant of such a Nanobody.
- A Nanobody in accordance with XLIII), which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than the EC50 value of Nanobody VHH 3E (SEQ ID NO:4) of WO 04/041862 in the same assay; or a humanized variant of such a Nanobody.
- A Nanobody in accordance with XLIII), which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 5nM; or a humanized variant of such a Nanobody.
- A Nanobody in accordance with XLIII), which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 3nM; or a humanized variant of such a Nanobody.
- A Nanobody in accordance with XLIII), which is chosen from the group consisting of SEQ ID NO's, 83 (TNF20), 85 (TNF21), 85 (TNF22), 96 (TNF23) or 98 (TNF33)TNF 13 (SEQ ID NO: 76), TNF 14 (SEQ ID NO: 77), TNF 29 (SEQ ID NO: 95) and TNF 30 (SEQ ID NO:96)

with some other preferred aspects being:

XLIV) A protein or polypeptide, which comprises or essentially consists of a Nanobody in accordance with XLII) or XLIII).

XLV) A protein or polypeptide, which comprises or essentially consists of at least one Nanobody in accordance with XLII) or XLIII).

XLVI) A protein or polypeptide, which comprises two Nanobodies in accordance with XLII) or XLIII).

XLVII) A protein or polypeptide, which comprises two Nanobodies in accordance with XLII) or XLIII), and which is such that said protein or polypeptide, upon binding to a TNF trimer, is capable inhibiting or reducing the TNF receptor crosslinking that is mediated by said TNF trimer and/or the signal transduction that is mediated by such receptor crosslinking..

XLVIII) A protein or polypeptide, which comprises two Nanobodies in accordance with XLII) or XLIII), and which is capable of intramolecular binding to at least two TNF receptor binding sites on a TNF trimer.

- A protein or polypeptide in accordance with any one of XLIV) or XLVIII),which comprises or essentially consists of the polypeptide TNF 6 (SEQ ID NO: 72) or TNF 9 (SEQ ID NO: 75, in which both Nanobodies TNF 3 have been humanized
- A protein or polypeptide in accordance with any one of XLIV) or XLVIII), which is pegylated.
- A protein or polypeptide, which comprises two Nanobodies in accordance with XLII) or XLIII), and which is such that said protein or polypeptide, upon binding to a TNF trimer, is capable inhibiting or reducing the TNF receptor crosslinking that is mediated by said TNF trimer and/or the signal transduction that is mediated by such receptor crosslinking; and/or which is such that said protein or polypeptide is capable of intramolecular binding to at least two TNF receptor binding sites on a TNF trimer, and which protein or polypeptide further comprises at least one Nanobody directed against human serum albumin.
- A protein or polypeptide in accordance with any one of XLIV) or XLVIII), in which the at least one Nanobody directed against human serum albumin is a humanized Nanobody.
- A protein or polypeptide in accordance with any one of XLIV) or XLVIII), in which the at least one Nanobody directed against human serum albumin is a humanized variant of the Nanobody ALB 1 (SEQ ID NO: 63).
- A protein or polypeptide in accordance with any one of XLIV) or XLVIII), in which the at least one Nanobody directed against human serum albumin is a chosen from the group consisting of ALB 3 (SEQ ID NO: 87), ALB 4 (SEQ ID NO: 88), ALB 5 (SEQ ID NO: 89), ALB 6 (SEQ ID NO: 100), ALB 7 (SEQ ID NO: 101), ALB 8 (SEQ ID NO: 102) ALB 9 (SEQ ID NO: 103) and ALB 10 (SEQ ID NO: 104).
- A protein or polypeptide in accordance with any one of XLIV) or XLVIII), in which the at least one Nanobody directed against human serum albumin is ALB 8.
- A protein or polypeptide in accordance with any one of XLIV) or XLVIII), which comprises or essentially consists of two humanized Nanobodies in accordance with any one of XLIV) or XLVIII) and one humanized variant of the Nanobody ALB 1 (SEQ ID NO: 63).

- A protein or polypeptide in accordance with any one of XLIV) or XLVIII), which comprises or essentially connsists of the polypeptide TNF26 (SEQ ID NO: 92), in which both the Nanobodies TNF 3 as well as the Nanobody ALB 1 has been humanized.

It should be noted that when a Nanobody is mentioned above as being "*in accordance with XLII" or "in accordance with XLIII"*, it is at least according to one of XLII) and/or XLIII), and may also include any one or more of the other aspects that are indicated as being "in accordance with XLII)" or "in accordance with XLIII)" above. Similarly, when a protein or polypeptide is mentioned above as being "*in accordance with any one of XLIV) or XLVIII)*", it is at least according to one of XL) to XLI), may be according to two or more of XLIV) to XLVIII), and may also include any one or more of the other aspects that are indicated as being "in accordance with any one of XLIV) or XLVIII) above.

For the Nanobodies based on Nanobody TNF3 above (including but not limited to the humanized Nanobodies), the framework sequences may generally be as described herein, and preferably are as follows:

a) FR1 comprises or is:

- the amino acid sequence of SEQ ID NO: 138;or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 138; or
- an amino acid sequences that has only 1 amino acid difference with the amino acid sequence of SEQ ID NO: 138;

and

b) FR2 comprises or is:

- the amino acid sequence of SEQ ID NO: 206; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 206; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence of SEQ ID NO: 206;

and

c) FR3 comprises or is:

- the amino acid sequence of SEQ ID NO: 274; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 274; or
- an amino acid sequences that has only 1 amino acid difference with the amino acid sequence of SEQ ID NO: 274.

and

d) FR4 comprises or is:

- the amino acid sequence of SEQ ID NO: 342; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 342; or
- an amino acid sequences that has only 1 amino acid difference with the amino acid sequence of SEQ ID NO: 342;

in which the amino acid differences present in the framework sequences are more preferably as described herein.

In another aspect, the invention relates to a Nanobody with an amino acid sequence that is chosen from the group consisting of SEQ ID NO's: 52 to 60, from the group consisting of SEQ ID NO's: 76 to 86, from the group consisting of SEQ ID NO's: 95 to 99, from the group consisting of SEQ ID NO's 105 to 129 or from the group consisting of from amino acid sequences that have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more "sequence identity" (as defined herein) with one or more of the amino acid sequences of SEQ ID NO's: 52 to 60, SEQ ID NO's: 76 to 86, SEQ ID NO's: 95 to 99 or SEQ ID NO's 105 to 129, in which the latter amino acid sequences most preferably have framework sequences that are as further defined below under the general description of the framework sequences of Nanobodies.

According to a specific, but non-limiting embodiment, the latter amino acid sequences are "humanized", as further described herein.

Most preferably, the Nanobodies of the invention are chosen from the group consisting of SEQ ID NO's: 52 to 60, from the group consisting of SEQ ID NO's: 76 to 86, from the group consisting of SEQ ID NO's: 95 to 99, or from the group consisting of SEQ ID NO's 105 to 129, of which the "humanized" Nanobodies of SEQ ID NO's 76 to 86 and SEQ ID NO's: 95 to 99 may be particularly preferred.

As mentioned above, a particularly preferred Nanobody of the invention is the clone PMP1C2 (TNF1; SEQ ID NO: 52). Thus, in a preferred aspect, the invention relates to a Nanobody with an amino acid sequence that is chosen from the group consisting of SEQ ID NO: 52 or from the group consisting of from amino acid sequences that have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more "sequence identity" (as defined herein) with the amino acid sequence of SEQ ID NO:52, in which the latter amino acid sequences most preferably have framework sequences that are as further defined below under the general description of the framework sequences of Nanobodies.

Particularly preferred are humanized variants of the clone PMP1C2 (TNF1; SEQ ID NO: 52). Some preferred, but non-limiting examples of such humanized variants are the clones TNF13 (SEQ ID NO: 76), TNF14 (SEQ ID NO:77), TNF29 (SEQ ID NO: 95) and TNF 30 (SEQ ID NO: 96). Thus, in a preferred aspect, the invention relates to a humanized Nanobody with an amino acid sequence that is chosen from the group consisting of SEQ ID NO's: 76, 77, 95 or 96, or from the group consisting of from amino acid sequences that have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more "sequence identity" (as defined herein) with one of the amino acid sequences of SEQ ID NO's: 76, 77, 95 or 96, in which the latter amino acid sequences most preferably have framework sequences that are as further defined below under the general description of the framework sequences of Nanobodies.

According to one preferred embodiment, the Nanobody of the invention is a humanized variant of the Nanobody TNF 1 (SEQ ID NO: 52).

Some preferred aspects of this embodiment of the invention are:

XLIX) A humanized variant of the Nanobody TNF 1, which has a Koff rate for TNF of better than 2.10-3 (1/s), preferably better than 1.10-3 (1/s).

L) A humanized variant of the Nanobody TNF 1, which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than the EC50 value of Nanobody VHH 3E (SEQ ID NO:4) of WO 04/041862 in the same assay.

LI) A humanized variant of the Nanobody TNF 1, which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 5nM.

LII) A humanized variant of the Nanobody TNF 1, which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 3nM.

LIII) A protein or polypeptide, which comprises or essentially consists of at least one Nanobody which is a humanized variant of the Nanobody TNF 1 in accordance with any one of XLIX) to LII)

LIV) A protein or polypeptide, which comprises or essentially consists of two Nanobodies which are humanized variants of the Nanobody TNF 1 in accordance with any one of XLIX) to LII) (optionally linked via a linker).

LV) A protein or polypeptide, which comprises or essentially consists of two Nanobodies which are humanized variants of the Nanobody TNF 1 in accordance with any one of XLIX) to LII) (optionally linked via a linker), and which is such that said protein or polypeptide, upon binding to a TNF trimer, is capable inhibiting or reducing the TNF receptor crosslinking that is mediated by said TNF trimer and/or the signal transduction that is mediated by such receptor crosslinking..

LVI) A protein or polypeptide, which comprises or essentially consists of two Nanobodies which are humanized variants of the Nanobody TNF 1 in accordance with any one of XLIX) to LII) and which is capable of intramolecular binding to at least two TNF receptor binding sites on a TNF trimer.

LVII)A protein or polypeptide which comprises or essentially consists of the polypeptide TNF 7 (SEQ ID NO: 73), in which both Nanobodies TNF 1 have been humanized.

LVIII) A protein or polypeptide which comprises or essentially consists of the polypeptide TNF 7 (SEQ ID NO: 73), in which both Nanobodies TNF 1 have been humanized, and which is pegylated.

LIX) A protein or polypeptide, which comprises or essentially consists of two Nanobodies which are humanized variants of the Nanobody TNF 1 in accordance with any one of XLIX) to LII), and which further comprises at least one Nanobody directed against human serum albumin.

LX) A protein or polypeptide, which comprises or essentially consists of two Nanobodies which are humanized variants of the Nanobody TNF 1 in accordance with any one of XLIX) to LII), which are each linked (optionally linked via a linker) to one Nanobody directed against human serum albumin.

- A protein or polypeptide in accordance with any one of LIII) to LX), in which the at least one Nanobody directed against human serum albumin is a humanized Nanobody.
- A protein or polypeptide in accordance with any one of LIII) to LX), in which the at least one Nanobody directed against human serum albumin is a humanized variant of the Nanobody ALB 1 (SEQ ID NO: 63).
- A protein or polypeptide in accordance with any one of LIII) to LX), in which the at least one Nanobody directed against human serum albumin is a chosen from the group consisting of ALB 3 (SEQ ID NO: 87), ALB 4 (SEQ ID NO: 88), ALB 5 (SEQ ID NO: 89), ALB 6 (SEQ ID NO: 100), ALB 7 (SEQ ID NO: 101), ALB 8 (SEQ ID NO: 102) ALB 9 (SEQ ID NO: 103) and ALB 10 (SEQ ID NO: 104).
- A protein or polypeptide in accordance with any one of LIII) to LX), in which the at least one Nanobody directed against human serum albumin is ALB 8.
- A protein or polypeptide in accordance with any one of LIII) to LX), which comprises or essentially consists of the polypeptide TNF24 (SEQ ID NO: 90), in which both the Nanobody TNF 1 as well as the Nanobody ALB TNF 1 has been humanized.
- A protein or polypeptide in accordance with any one of LIII) to LX), which comprises or essentially consists of two Nanobodies TNF 30 and one Nanobody ALB 8.

According to one preferred embodiment, the Nanobody of the invention is a humanized variant of the Nanobody TNF 3 (SEQ ID NO: 60).

Some preferred aspects of this embodiment of the invention are:

LXI) A humanized variant of the Nanobody TNF 3, which has a Koff rate for TNF of better than 2.10-3 (1/s), preferably better than 1.10-3 (1/s).

LXII) A humanized variant of the Nanobody TNF 3, which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than the EC50 value of Nanobody VHH 3E (SEQ ID NO:4) of WO 04/041862 in the same assay.

LXIII) A humanized variant of the Nanobody TNF 3, which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 5nM.

LXIV) A humanized variant of the Nanobody TNF 3, which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 3nM.

LXV) A protein or polypeptide, which comprises or essentially consists of at least one Nanobody which is a humanized variant of the Nanobody TNF 3 in accordance with any one of LXI) to LXIV)

LXVI) A protein or polypeptide, which comprises or essentially consists of two Nanobodies which are humanized variants of the Nanobody TNF 3 in accordance with any one of LXI) to LXIV) (optionally linked via a linker).

LXVII) A protein or polypeptide, which comprises or essentially consists of two Nanobodies which are humanized variants of the Nanobody TNF 3 in accordance with any one of LXI) to LXIV) (optionally linked via a linker), and which is such that said protein or polypeptide, upon binding to a TNF trimer, is capable inhibiting or reducing the TNF receptor crosslinking that is mediated by said TNF trimer and/or the signal transduction that is mediated by such receptor crosslinking..

LXVIII) A protein or polypeptide, which comprises or essentially consists of two Nanobodies which are humanized variants of the Nanobody TNF 3 in accordance with any one of LXI) to LXIV) and which is capable of intramolecular binding to at least two TNF receptor binding sites on a TNF trimer.

LXIX) A protein or polypeptide which comprises or essentially consists of the polypeptide TNF 6 (SEQ ID NO: 72) or TNF 9 (SEQ ID NO: 75), in which both Nanobodies TNF 3 have been humanized.

LXX) A protein or polypeptide which comprises or essentially consists of the polypeptide TNF 6 (SEQ ID NO: 72) or TNF 9 (SEQ ID NO: 75), in which both Nanobodies TNF 3 have been humanized, and which is pegylated.

LXXI) A protein or polypeptide, which comprises or essentially consists of two Nanobodies which are humanized variants of the Nanobody TNF 3 in accordance with any one of LXI) to LXIV), and which further comprises at least one Nanobody directed against human serum albumin.

LXXII) A protein or polypeptide, which comprises or essentially consists of two Nanobodies which are humanized variants of the Nanobody TNF 3 in accordance with any one of LXI) to LXIV), which are each linked (optionally linked via a linker) to one Nanobody directed against human serum albumin.

- A protein or polypeptide in accordance with any one of LXV) to LXXII), in which the at least one Nanobody directed against human serum albumin is a humanized Nanobody.
- A protein or polypeptide in accordance with any one of LXV) to LXXII), in which the at least one Nanobody directed against human serum albumin is a humanized variant of the Nanobody ALB 1 (SEQ ID NO: 63).
- A protein or polypeptide in accordance with any one of LXV) to LXXII), in which the at least one Nanobody directed against human serum albumin is a chosen from the group consisting of ALB 3 (SEQ ID NO: 87), ALB 4 (SEQ ID NO: 88), ALB 5 (SEQ ID NO: 89), ALB 6 (SEQ ID NO: 100), ALB 7 (SEQ ID NO: 101), ALB 8 (SEQ ID NO: 102) ALB 9 (SEQ ID NO: 103) and ALB 10 (SEQ ID NO: 104).
- A protein or polypeptide in accordance with any one of LXV) to LXXII), in which the at least one Nanobody directed against human serum albumin is ALB 8.
- A protein or polypeptide in accordance with any one of LXV) to LXXII), which comprises or essentially consists of the polypeptide TNF26 (SEQ ID NO: 92), in which both the Nanobodies TNF 3 as well as the Nanobody ALB 1 has been humanized.

In another aspect, the invention relates to a polypeptide that comprises or essentially consists of at least one Nanobody against TNF-alpha as defined herein. Such polypeptides are also referred to herein as "polypeptides of the invention" and may be as further described hereinbelow and/or as generally described in WO 04/041862 for the Nanobodies disclosed therein, and may for example be multivalent polypeptides or multispecific polypeptides, again as further described hereinbelow.

Preferably, a polypeptide of the invention is either bivalent or trivalent (i.e. comprising two or three Nanobodies of the invention, respectively, optionally linked via one or two linkers as defined below, respectively) or a multispecific polypeptide, comprising one or two, and preferably two, Nanobodies of the invention and at least one Nanobody directed against a serum protein, and in particular against a human serum protein, such as against human serum albumin.

In one preferred, but non-limiting embodiments, the Nanobodies of the invention present in the polypeptides of the invention are chosen from the group consisting of SEQ ID NO's: 52 to 60 and SEQ ID NO's 105-129 or from humanized variants thereof, and in particular from the "humanized" Nanobodies of SEQ ID NO's 76 to 86 and SEQ ID NO's:

95 to 99. The Nanobodies against human serum albumin present in the polypeptides of the invention are preferably as defined below, and are more preferably chosen from the group consisting of SEQ ID NO's: 61 to 67, SEQ ID NO's: 87 to 89 and SEQ ID NO's: 100-104, and in particular from the "humanized" Nanobodies against human serum albumin of SEQ ID NO's 76 to 86 and SEQ ID NO's 100-104.

With respect to the Nanobodies that are present in the polypeptides of the invention, it will be clear to the skilled person that the Nanobodies that are mentioned herein as "preferred" (or as "more preferred", "even more preferred", etc.) are also preferred (or more preferred, or even more preferred, etc.) for use in the polypeptides described herein. Thus, polypeptides that comprise or essentially consist of one or more "preferred" Nanobodies of the invention will generally be preferred, and polypeptides that comprise or essentially consist of one or more "more preferred" Nanobodies of the invention of the invention will generally be more preferred, etc..

Thus, in the invention, polypeptides that comprise one or more Nanobodies that essentially consist of one of the preferred variants of clone PMP1C2 (TNF1; SEQ ID NO: 52), in which said preferred variants are as defined herein, are particularly preferred. Even more preferred are polypeptides that comprise one or more Nanobodies that essentially consist of one of the humanized variants of clone PMP1C2 (TNF1; SEQ ID NO: 52), in which said humanized variants are as defined herein (examples being, without limitation, TNF13, TNF14, TNF29 and TNF30). TNF30 is a particularly preferred humanized "building block" for use in the polypeptides of the invention.

Some preferred, but non-limiting examples of such proteins and polypeptides are PMP1C2 itself, the humanized variants TNF13, TNF14, TNF29 and TNF30; the constructs of SEQ ID NO: 70 (TNF4), SEQ ID NO: 73 (TNF7), SEQ ID NO: 90 (TNF24), SEQ ID NO: 93 (TNF27); and the constructs of SEQ ID NO: 417 (TNF60), SEQ ID NO: 419 (TNF55) and SEQ ID NO: 420 (TNF56), in which the latter three constructs contain the humanized variant TNF 30 as a building block.

As mentioned herein, the Nanobodies and constructs described herein may be pegylated, or contain one or more (additional) amino acid residues that allow for pegylation and/or facilitate pegylation. Two preferred, but non-limiting examples of such polypeptides are TNF55 and TNF56, which both contain an additional cysteine residue for easy attachment of a PEG-group.

Some preferred, but non-limiting examples of polypeptides of the invention are the bivalent polypeptides of the invention of SEQ ID NO's: 70 to 75 and the multispecific polypeptides of the invention of SEQ ID NO's: 90 to 94 and SEQ ID NO's 417 to 420.

As can be seen from the data represented below, and in particular from the data given in the Comparative Example, the Nanobodies and/or polypeptides of the invention have improved properties. In particular, the proteins and polypeptides of the invention may havean improved affinity for human TNF-alpha (expressed as the EC_{50}-value in the KYM assay described herein), compared to the commercially available anti-TNF biologicals Enbrel™, Humira™ and Remicade™. Also, the Nanobodies described herein may have an improved affinity for TNF-alpha compared to best performing Nanobody described in the International application WO 04/041862. It can thus be expected that polypeptides of the invention comprising at least one of the Nanobodies of the invention will also have improved properties compared to polypeptides that comprise only the Nanobodies against TNF-alpha described in WO 04/041862.

More in particular, a polypeptide as described herein that comprises two or more (and preferably two) Nanobodies as herein (and optionally for example a Nanobody against human serum albumin), has an an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than the EC50 value of Humira® en Remicade ®, and preferably also better than Enbrel® in the same assay.

For example, such a protein or polypeptide preferably has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 0.2 nM, preferably better than 0.1 nM, such as betet than 0.7 Nm and in particular better than 0.4 nM.

It has also been shown by applicants that Nanobodies against mouse TNF-alpha and polypeptides comprising Nanobodies against mouse TNF-alpha show a beneficial biological activity in the following disease models (data not shown):

- The dextran sulfate sodium ("DSS") model of colitis, using both regular mice as well as IL-10 knock out mice, as described by Okayasu *et al.* (*Gastroenterol* 1990, 98(3): 694)
- The collagen induced arthritis ("CIA") model of arthritis ("RA"), as described by Courtenay *et al.* (*Nature* 1980, 283(5748): 666), using both regular mice as well as IL-10 knock out mice;

- The IL-10 knockout mice model of IBD, as for example described by Rennick *et al.* (*Clin Immunol Immunopathol* 1995, 76(3 Pt 2): S174)
- The Kollias model of RA as for example described by Keffer *et al.* (*EMBO J* 1991, 10(13): 4025)
- The 2,4,6-trinitrobenzenesulphonic acid ("TNBS") model of IBD, as described by Elson *et al.* (*J Immunol* 1996, 157(5): 2174)
- The CIA model of RA, described by Koppieters et al (manuscript in preparation);
- The synovial-derived fibroblast model (described below); and
- The murine air pouch model.

Preferably, the Nanobodies described herein are better than Nanobody 1A from WO 04/041862 in at least one of these models, and preferably in all of these models; and more p are better than Nanobody 3E from WO 04/041862 in at least one of these models, and preferably in all of these models. Also, the polypeptides described herein are preferably equivalent to or better than Humira® or Remicade ® in at least one of these models, and preferably in all of these models; and more preferably also equivalent to or better than Enbrel® in at least one of these models, and preferably in all of these models.

These data confirm that Nanobodies against TNF-alpha and polypeptides containing the same, such as the Nanobodies and polypeptides described in WO 04/041862 and in particular the Nanobodies and polypeptides described herein, should have therapeutic efficacy against TNF mediated diseases and disorders, such as the diseases and disorders mentioned above.

In another aspect, the invention relates to a nucleic acid that encodes a Nanobody of the invention and/or a polypeptide of the invention. Such a nucleic acid will also be referred to below as a "nucleic acid of the invention" and may for example be in the form of a genetic construct, as defined below.

In another aspect, the invention relates to host or host cell that expresses or is capable of expressing a Nanobody of the invention and/or a polypeptide of the invention; and/or that contains a nucleic acid encoding a Nanobody of the invention and/or a polypeptide of the invention. Such a host or a host cell may also be analogous to the hosts and host cells described in WO 04/041862, but expressing or capable of expressing a Nanobody of the invention and/or a polypeptide of the invention and/or containing a nucleic acid as described herein.

The invention further relates to a product or composition containing or comprising a Nanobody of the invention, a polypeptide of the invention; and/or a nucleic acid of the invention. Such a product or composition may for example be a pharmaceutical composition (as described below) or a product or composition for diagnostic use (as also described below). Such a product or composition may also be analogous to the products and compositions described in WO 04/041862, but containing or comprising a Nanobody of the invention, a polypeptide of the invention or a nucleic acid of the invention.

The invention further relates to methods for preparing or generating the Nanobodies, polypeptides, nucleic acids, host cells, products and compositions as described herein, which methods are as further described below. Also, generally, the Nanobodies, polypeptides, nucleic acids, host cells, products and compositions described herein may also be prepared and used in a manner analogous to the manner described in WO 04/041862.

The invention further relates to applications and uses of the above Nanobodies, polypeptides, nucleic acids, host cells, products and compositions described herein, which applications and uses include, but are not limited to, the applications and uses described hereinbelow and/or the further uses and applications for Nanobodies against TNF-alpha and/or for polypeptides containing the same in WO 04/041862.

Other aspects, embodiments, advantages and applications of the invention will become clear from the further description hereinbelow.

Detailed description of the invention

The above and other aspects and embodiments of the invention will become clear from the further description hereinbelow, in which:

a) Unless indicated or defined otherwise, all terms used have their usual meaning in the art, which will be clear to the skilled person. Reference is for example made to the standard handbooks, such as Sambrook et al, "Molecular Cloning: A Laboratory Manual" (2nd.Ed.), Vols. 1-3, Cold Spring Harbor Laboratory Press (1989); F. Ausubel et al, eds., "Current protocols in molecular biology", Green Publishing and Wiley Interscience, New York (1987); Lewin, "Genes II", John Wiley & Sons, New York, N.Y., (1985); Old et al., "Principles of Gene Manipulation: An Introduction to Genetic Engineering", 2nd edition, University of California Press, Berkeley, CA (1981); Roitt et al., "Immunology" (6th. Ed.), Mosby/Elsevier, Edinburgh (2001); Roitt et al., Roitt's Essential Immunology, 10th

Ed. Blackwell Publishing, UK (2001); and Janeway et al., "Immunobiology" (6th Ed.), Garland Science Publishing/Churchill Livingstone, New York (2005), as well as to the general background art cited herein;

b) Unless indicated otherwise, the term "*immunoglobulin sequence*" - whether it used herein to refer to a heavy chain antibody or to a conventional 4-chain antibody - is used as a general term to include both the full-size antibody, the individual chains thereof, as well as all parts, domains or fragments thereof (including but not limited to antigen-binding domains or fragments such as V_{HH} domains or V_H/V_L domains, respectively). In addition, the term "*sequence*" as used herein (for example in terms like "immunoglobulin sequence", "antibody sequence", "variable domain sequence", "V_{HH} sequence" or "protein sequence"), should generally be understood to include both the relevant amino acid sequence as well as nucleic acid sequences or nucleotide sequences encoding the same, unless the context requires a more limited interpretation;

c) Unless indicated otherwise, all methods, steps, techniques and manipulations that are not specifically described in detail can be performed and have been performed in a manner known per se, as will be clear to the skilled person. Reference is for example again made to the standard handbooks, to the general background art referred to above and to the further references cited therein;

d) Amino acid residues will be indicated according to the standard three-letter or one-letter amino acid code, as mentioned in Table 1;

Table 1: one-letter and three-letter amino acid code

Nonpolar, uncharged (at pH 6.0 – 7.0)[3]	Alanine	Ala	A
	Valine	Val	V
	Leucine	Leu	L
	Isoleucine	Ile	I
	Phenylalanine	Phe	F
	Methionine[1]	Met	M
	Tryptophan	Trp	W
	Proline	Pro	P
Polar, uncharged (at pH 6.0-7.0)	Glycine[2]	Gly	G
	Serine	Ser	S
	Threonine	Thr	T
	Cysteine	Cys	C
	Asparagine	Asn	N
	Glutamine	Gln	Q
	Tyrosine	Tyr	Y
Polar, charged (at pH 6.0-7.0)	Lysine	Lys	K
	Arginine	Arg	R
	Histidine[4]	His	H
	Aspartate	Asp	D
	Glutamate	Glu	E

Notes:

(1) Sometimes also considered to be a polar uncharged amino acid.

(2) Sometimes also considered to be a nonpolar uncharged amino acid.

(3) As will be clear to the skilled person, the fact that an amino acid residue is referred to in this Table as being either charged or uncharged at pH 6.0 to 7.0 does not reflect in any way on the charge said amino acid residue may have at a pH lower than 6.0 and/or at a pH higher than 7.0; the amino acid residues mentioned in the Table can be either charged and/or uncharged at such a higher or lower pH, as will be clear to the skilled person.

(4) As is known in the art, the charge of a His residue is greatly dependant upon even small shifts in pH, but a His residu can generally be considered essentially uncharged at a pH of about 6.5.

e) For the purposes of comparing two or more nucleotide sequences, the percentage of "*sequence identity*" between a first nucleotide sequence and a second nucleotide sequence may be calculated by dividing [*the number of nucleotides in the first nucleotide sequence that are identical to the nucleotides at the corresponding positions in the second nucleotide sequence*] by [*the total number of nucleotides in the first nucleotide sequence*] and multiplying by [100%], in which each deletion, insertion, substitution or addition of a nucleotide in the second nucleotide sequence - compared to the first nucleotide sequence - is considered as a difference at a single nucleotide (position).

Alternatively, the degree of sequence identity between two or more nucleotide sequences may be calculated using a known computer algorithm for sequence alignment such as NCBI Blast v2.0, using standard settings.

Some other techniques, computer algorithms and settings for determining the degree of sequence identity are for example described in WO 04/037999, EP 0 967 284, EP 1 085 089, WO 00/55318, WO 00/78972, WO 98/49185 and GB 2 357 768-A.

Usually, for the purpose of determining the percentage of "sequence identity" between two nucleotide sequences in accordance with the calculation method outlined hereinabove, the nucleotide sequence with the greatest number of nucleotides will be taken as the "first" nucleotide sequence, and the other nucleotide sequence will be taken as the "second" nucleotide sequence;

f) For the purposes of comparing two or more amino acid sequences, the percentage of "*sequence identity*" between a first amino acid sequence and a second amino acid sequence may be calculated by dividing [*the number of amino acid residues in the first amino acid sequence that are identical to the amino acid residues at the corresponding positions in the second amino acid sequence*] by [*the total number of nucleotides in the first amino acid sequence*] and multiplying by [*100%*], in which each deletion, insertion, substitution or addition of an amino acid residue in the second amino acid sequence - compared to the first amino acid sequence - is considered as a difference at a single amino acid residue (position), i.e. as an "*amino acid difference*" as defined below.

Alternatively, the degree of sequence identity between two amino acid sequences may be calculated using a known computer algorithm, such as those mentioned above for determining the degree of sequence identity for nucleotide sequences, again using standard settings.

Usually, for the purpose of determining the percentage of "sequence identity" between two amino acid sequences in accordance with the calculation method outlined hereinabove, the amino acid sequence with the greatest number of amino acid residues will be taken as the "first" amino acid sequence, and the other amino acid sequence will be taken as the "second" amino acid sequence.

Also, in determining the degree of sequence identity between two amino acid sequences, the skilled person may take into account so-called "conservative" amino acid substitutions, which can generally be described as amino acid substitutions in which an amino acid residue is replaced with another amino acid residue of similar chemical structure and which has little or essentially no influence on the function, activity or other biological properties of the polypeptide. Such conservative amino acid substitutions are well known in the art, for example from WO 04/037999, GB-A-2 357 768, WO 98/49185, WO 00/46383 and WO 01/09300; and (preferred) types and/or combinations of such substitutions may be selected on the basis of the pertinent teachings from WO 04/037999 as well as WO 98/49185 and from the further references cited therein.

Such conservative substitutions preferably are substitutions in which one amino acid within the following groups (a) – (e) is substituted by another amino acid residue within the same group: (a) small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro and Gly; (b) polar, negatively charged residues and their (uncharged) amides: Asp, Asn, Glu and Gln; (c) polar, positively charged residues: His, Arg and Lys; (d) large aliphatic, nonpolar residues: Met, Leu, Ile, Val and Cys; and (e) aromatic residues: Phe, Tyr and Trp.

Particularly preferred conservative substitutions are as follows: Ala into Gly or into Ser; Arg into Lys; Asn into Gln or into His; Asp into Glu; Cys into Ser; Gln into Asn; Glu into Asp; Gly into Ala or into Pro; His into Asn or into Gln; Ile into Leu or into Val; Leu into Ile or into Val; Lys into Arg, into Gln or into Glu; Met into Leu, into Tyr or into Ile; Phe into Met, into Leu or into Tyr; Ser into Thr; Thr into Ser; Trp into Tyr; Tyr into Trp; and/or Phe into Val, into Ile or into Leu.

Any amino acid substitutions applied to the polypeptides described herein may also be based on the analysis of the frequencies of amino acid variations between homologous proteins of different species developed by Schulz et al., Principles of Protein Structure, Springer-Verlag, 1978, on the analyses of structure forming potentials developed by Chou and Fasman, Biochemistry 13: 211, 1974 and Adv. Enzymol., 47: 45-149, 1978, and on

the analysis of hydrophobicity patterns in proteins developed by Eisenberg et al., Proc. Nad. Acad Sci. USA 81: 140-144, 1984; Kyte & Doolittle; J Molec. Biol. 157: 105-132, 198 1, and Goldman et al., Ann. Rev. Biophys. Chem. 15: 321-353, 1986, all incorporated herein in their entirety by reference. Information on the primary, secondary and tertiary structure of Nanobodies given in the description herein and in the general background art cited above. Also, for this purpose, the crystal structure of a VHH domain from a llama is for example given by Desmyter et al., Nature Structural Biology, Vol. 3, 9, 803 (1996); Spinelli et al., Natural Structural Biology (1996); 3, 752-757; and Decanniere et al., Structure, Vol. 7, 4, 361 (1999). Further information about some of the amino acid residues that in conventional VH domains form the VH/VL interface and potential camelizing substitutions on these positions can be found in the prior art on Nanobodies cited herein;

g) amino acid sequences and nucleic acid sequences are said to be "*exactly the same*" if they have 100% sequence identity (as defined herein) over their entire length;

h) when comparing two amino acid sequences, the term "*amino acid difference*" refers to an insertion, deletion or substitution of a single amino acid residue on a position of the first sequence, compared to the second sequence; it being understood that two amino acid sequences can contain one, two or more such amino acid differences;

i) a nucleic acid sequence or amino acid sequence is considered to be "*(in) essentially isolated (form)*" - for example, compared to its native biological source and/or the reaction medium or cultivation medium from which it has been obtained - when it has been separated from at least one other component with which it is usually associated in said source or medium, such as another nucleic acid, another protein/polypeptide, another biological component or macromolecule or at least one contaminant, impurity or minor component. In particular, a nucleic acid sequence or amino acid sequence is considered "*essentially isolated*" when it has been purified at least 2-fold, in particular at least 10-fold, more in particular at least 100-fold, and up to 1000-fold or more. A nucleic acid sequence or amino acid sequence that is "in essentially isolated form" is preferably essentially homogeneous, as determined using a suitable technique, such as a suitable chromatographical technique, such as polyacrylamide-gelelectrophoresis;

j) The term "domain" as used herein generally refers to a globular region of an antibody chain, and in particular to a globular region of a heavy chain antibody, or to a polypeptide that essentially consists of such a globular region. Usually, such a domain will

comprise peptide loops (for example 3 or 4 peptide loops) stabilized, for example, as a sheet or by disulfide bonds.

k) The term 'antigenic determinant' refers to the epitope on the antigen recognized by the antigen-binding molecule (such as a Nanobody or a polypeptide of the invention) and more in particular by the antigen-binding site of said molecule. The terms "antigenic determinant" and "epitope' may also be used interchangeably herein.

l) An amino acid sequence (such as a Nanobody, an antibody, a polypeptide of the invention, or generally an antigen binding protein or polypeptide or a fragment thereof) that can bind to, that has affinity for and/or that has specificity for a specific antigenic determinant, epitope, antigen or protein (or for at least one part, fragment or epitope thereof) is said to be "against" or "directed against" said antigenic determinant, epitope, antigen or protein.

m) The term "specificity" refers to the number of different types of antigens or antigenic determinants to which a particular antigen-binding molecule or antigen-binding protein (such as a Nanobody or a polypeptide of the invention) molecule can bind. The specificity of an antigen-binding protein can be determined based on affinity and/or avidity. The affinity, represented by the equilibrium constant for the dissociation of an antigen with an antigen-binding protein (KD), is a measure for the binding strength between an antigenic determinant and an antigen-binding site on the antigen-binding protein: the lesser the value of the KD, the stronger the binding strength between an antigenic determinant and the antigen-binding molecule (alternatively, the affinity can also be expressed as the affinity constant (KA), which is 1/KD). As will be clear to the skilled person (for example on the basis of the further disclosure herein), affinity can be determined in a manner known per se, depending on the specific antigen of interest. Avidity is the measure of the strength of binding between an antigen-binding molecule (such as a Nanobody or polypeptide of the invention) and the pertinent antigen. Avidity is related to both the affinity between an antigenic determinant and its antigen binding site on the antigen-binding molecule and the number of pertinent binding sites present on the antigen-binding molecule. Typically, antigen-binding proteins (such as the Nanobodies and/or polypeptides of the invention) will bind with a dissociation constant (KD) of 10^{-5} to 10^{-12} moles/liter (M) or less, and preferably 10^{-7} to 10^{-12} moles/liter (M) or less and more preferably 10^{-8} to 10^{-12} moles/liter, and/or with an association constant(K_A) of at least 10^7 M^{-1}, preferably at least 10^8 M^{-1}, more preferably at least 10^9 M-1, such as at least 10^{12} M^{-1}.

Any K_D value greater than 10^{-4} M is generally considered to indicate non-specific binding. Preferably, a Nanobody or polypeptide of the invention will bind to the desired antigen with an K_D less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM. Specific binding of an antigen-binding protein to an antigen or antigenic determinant can be determined in any suitable manner known per se, including, for example, Scatchard analysis and/or competitive binding assays, such as radioimmunoassays (RIA), enzyme immunoassays (EIA) and sandwich competition assays, and the different variants thereof known per se in the art.

n) as further described hereinbelow, the amino acid sequence and structure of a Nanobody can be considered - without however being limited thereto - to be comprised of four framework regions or "FR's", which are referred to in the art and hereinbelow as "*Framework region 1*" or "*FR1*"; as "*Framework region 2*" or"*FR2*"; as "*Framework region 3*" or "*FR3*"; and as "*Framework region 4*" or "*FR4*", respectively; which framework regions are interrupted by three complementary determining regions or "CDR's", which are referred to in the art as "*Complementarity Determining Region 1*"or "*CDR1*"; as "*Complementarity Determining Region 2*" or "*CDR2*"; and as "*Complementarity Determining Region 3*" or "*CDR3*", respectively;

o) as also further describe hereinbelow, the total number of amino acid residues in a Nanobody can be in the region of 110-120, is preferably 112-115, and is most preferably 113. It should however be noted that parts, fragments or analogs (as further described hereinbelow) of a Nanobody are not particularly limited as to their length and/or size, as long as such parts, fragments or analogs meet the further requirements outlined hereinbelow and are also preferably suitable for the purposes described herein;

p) the amino acid residues of a Nanobody are numbered according to the general numbering for V_H domains given by Kabat et al. ("*Sequence of proteins of immunological interest*", US Public Health Services, NIH Bethesda, MD, Publication No. 91), as applied to V_{HH} domains from Camelids in the article of Riechmann and Muyldermans, referred to above (see for example Figure 2 of said reference). According to this numbering, FR1 of a Nanobody comprises the amino acid residues at positions 1-30, CDR1 of a Nanobody comprises the amino acid residues at positions 31-36, FR2 of a Nanobody comprises the amino acids at positions 36-49, CDR2 of a Nanobody comprises the amino acid residues at positions 50-65, FR3 of a Nanobody comprises the amino acid residues at positions 66-94, CDR3 of a Nanobody comprises the amino acid residues at positions 95-102, and FR4 of a

Nanobody comprises the amino acid residues at positions 103-113. [In this respect, it should be noted that - as is well known in the art for V_H domains and for V_{HH} domains - the total number of amino acid residues in each of the CDR's may vary and may not correspond to the total number of amino acid residues indicated by the Kabat numbering (that is, one or more positions according to the Kabat numbering may not be occupied in the actual sequence, or the actual sequence may contain more amino acid residues than the number allowed for by the Kabat numbering). This means that, generally, the numbering according to Kabat may or may not correspond to the actual numbering of the amino acid residues in the actual sequence. Generally, however, it can be said that, according to the numbering of Kabat and irrespective of the number of amino acid residues in the CDR's, position 1 according to the Kabat numbering corresponds to the start of FR1 and vice versa, position 36 according to the Kabat numbering corresponds to the start of FR2 and vice versa, position 66 according to the Kabat numbering corresponds to the start of FR3 and vice versa, and position 103 according to the Kabat numbering corresponds to the start of FR4 and vice versa.].

Alternative methods for numbering the amino acid residues of V_H domains, which methods can also be applied in an analogous manner to V_{HH} domains from Camelids and to Nanobodies, are the method described by Chothia et al. (*Nature* 342, 877-883 (1989)), the so-called "*AbM definition*" and the so-called "*contact definition*". However, in the present description, claims and figures, the numbering according to Kabat as applied to V_{HH} domains by Riechmann and Muyldermans will be followed, unless indicated otherwise; and

q) the Figures, Sequence Listing and the Experimental Part/Examples are only given to further illustrate the invention and should not be interpreted or construed as limiting the scope of the invention and/or of the appended claims in any way, unless explicitly indicated otherwise herein.

For a general description of heavy chain antibodies and the variable domains thereof, reference is inter alia made to the following references, which are mentioned as general background art: WO 94/04678, WO 95/04079 and WO 96/34103 of the Vrije Universiteit Brussel; WO 94/25591, WO 99/37681, WO 00/40968, WO 00/43507, WO 00/65057, WO 01/40310, WO 01/44301, EP 1134231 and WO 02/48193 of Unilever; WO 97/49805, WO 01/21817, WO 03/035694, WO 03/054016 and WO 03/055527 of the Vlaams Instituut voor Biotechnologie (VIB); WO 03/050531 of Algonomics N.V. and

applicant; WO 01/90190 by the National Research Council of Canada; WO 03/025020 (= EP 1 433 793) by the Institute of Antibodies; as well as WO 04/041867, WO 04/041862, WO 04/041865, WO 04/041863, WO 04/062551 by applicant and the further published patent applications by applicant;

Hamers-Casterman et al., Nature 1993 June 3; 363 (6428): 446-8; Davies and Riechmann, FEBS Lett. 1994 Feb 21; 339(3): 285-90; Muyldermans et al., Protein Eng. 1994 Sep; 7(9): 1129-3; Davies and Riechmann, Biotechnology (NY) 1995 May; 13(5): 475-9; Gharoudi et al., 9th Forum of Applied Biotechnology, Med. Fac. Landbouw Univ. Gent. 1995; 60/4a part I: 2097-2100; Davies and Riechmann, Protein Eng. 1996 Jun; 9(6): 531-7; Desmyter et al., Nat Struct Biol. 1996 Sep; 3(9): 803-11; Sheriff et al., Nat Struct Biol. 1996 Sep; 3(9): 733-6; Spinelli et al., Nat Struct Biol. 1996 Sep; 3(9): 752-7; Arbabi Ghahroudi et al., FEBS Lett. 1997 Sep 15; 414(3): 521-6; Vu et al., Mol Immunol. 1997 Nov-Dec; 34(16-17): 1121-31; Atarhouch et al., Journal of Camel Practice and Research 1997; 4: 177-182; Nguyen et al., J. Mol. Biol. 1998 Jan 23; 275(3): 413-8; Lauwereys et al., EMBO J. 1998 Jul 1; 17(13): 3512-20; Frenken et al., Res Immunol. 1998 Jul-Aug;149(6):589-99; Transue et al., Proteins 1998 Sep 1; 32(4): 515-22; Muyldermans and Lauwereys, J. Mol. Recognit. 1999 Mar-Apr; 12 (2): 131-40; van der Linden et al., Biochim. Biophys. Acta 1999 Apr 12; 1431(1): 37-46.; Decanniere et al., Structure Fold. Des. 1999 Apr 15; 7(4): 361-70; Ngyuen et al., Mol. Immunol. 1999 Jun; 36(8): 515-24; Woolven et al., Immunogenetics 1999 Oct; 50 (1-2): 98-101; Riechmann and Muyldermans, J. Immunol. Methods 1999 Dec 10; 231 (1-2): 25-38; Spinelli et al., Biochemistry 2000 Feb 15; 39(6): 1217-22; Frenken et al., J. Biotechnol. 2000 Feb 28; 78(1): 11-21; Nguyen et al., EMBO J. 2000 Mar 1; 19(5): 921-30; van der Linden et al., J. Immunol. Methods 2000 Jun 23; 240 (1-2): 185-95; Decanniere et al., J. Mol. Biol. 2000 Jun 30; 300 (1): 83-91; van der Linden et al., J. Biotechnol. 2000 Jul 14; 80(3): 261-70; Harmsen et al., Mol. Immunol. 2000 Aug; 37(10): 579-90; Perez et al., Biochemistry 2001 Jan 9; 40(1): 74-83; Conrath et al., J. Biol. Chem. 2001 Mar 9; 276 (10): 7346-50; Muyldermans et al., Trends Biochem Sci. 2001 Apr;26(4):230-5; Muyldermans S., J. Biotechnol. 2001 Jun; 74 (4): 277-302; Desmyter et al., J. Biol. Chem. 2001 Jul 13 ;276 (28): 26285-90; Spinelli et al., J. Mol. Biol. 2001 Aug 3; 311 (1): 123-9; Conrath et al., Antimicrob Agents Chemother. 2001 Oct; 45 (10): 2807-12; Decanniere et al., J. Mol. Biol. 2001 Oct 26; 313(3): 473-8; Nguyen et al., Adv Immunol. 2001; 79: 261-96; Muruganandam et al., FASEB J. 2002 Feb; 16 (2): 240-2; Ewert et al., Biochemistry 2002

Mar 19; 41 (11): 3628-36; Dumoulin et al., Protein Sci. 2002 Mar; 11 (3): 500-15; Cortez-Retamozo et al., Int. J. Cancer. 2002 Mar 20; 98 (3): 456-62; Su et al., Mol. Biol. Evol. 2002 Mar; 19 (3): 205-15; van der Vaart JM., Methods Mol Biol. 2002; 178: 359-66; Vranken et al., Biochemistry 2002 Jul 9; 41 (27): 8570-9; Nguyen et al., Immunogenetics 2002 Apr; 54 (1): 39-47; Renisio et al., Proteins 2002 Jun 1; 47 (4): 546-55; Desmyter et al., J. Biol. Chem. 2002 Jun 28; 277 (26): 23645-50; Ledeboer et al., J. Dairy Sci. 2002 Jun; 85 (6): 1376-82; De Genst et al., J. Biol. Chem. 2002 Aug 16; 277 (33): 29897-907; Ferrat et al., Biochem. J. 2002 Sep 1; 366 (Pt 2): 415-22; Thomassen et al., Enzyme and Microbial Technol. 2002; 30: 273-8; Harmsen et al., Appl. Microbiol. Biotechnol. 2002 Dec; 60 (4): 449-54; Jobling et al., Nat Biotechnol. 2003 Jan; 21 (1): 77-80; Conrath et al., Dev. Comp. Immunol. 2003 Feb; 27 (2): 87-103; Pleschberger et al., Bioconjug. Chem. 2003 Mar-Apr; 14 (2): 440-8; Lah et al., J. Biol. Chem. 2003 Apr 18; 278 (16): 14101-11; Nguyen et al., Immunology. 2003 May; 109 (1): 93-101; Joosten et al., Microb. Cell Fact. 2003 Jan 30; 2 (1): 1; Li et al., Proteins 2003 Jul 1; 52 (1): 47-50; Loris et al., Biol Chem. 2003 Jul 25; 278 (30): 28252-7; van Koningsbruggen et al., J. Immunol. Methods. 2003 Aug; 279 (1-2): 149-61; Dumoulin et al., Nature. 2003 Aug 14; 424 (6950): 783-8; Bond et al., J. Mol. Biol. 2003 Sep 19; 332 (3): 643-55; Yau et al., J. Immunol. Methods. 2003 Oct 1; 281 (1-2): 161-75; Dekker et al., J. Virol. 2003 Nov; 77 (22): 12132-9; Meddeb-Mouelhi et al., Toxicon. 2003 Dec; 42 (7): 785-91; Verheesen et al., Biochim. Biophys. Acta 2003 Dec 5; 1624 (1-3): 21-8; Zhang et al., J Mol Biol. 2004 Jan 2; 335 (1): 49-56; Stijlemans et al., J Biol Chem. 2004 Jan 9; 279 (2): 1256-61; Cortez-Retamozo et al., Cancer Res. 2004 Apr 15; 64 (8): 2853-7; Spinelli et al., FEBS Lett. 2004 Apr 23; 564 (1-2): 35-40; Pleschberger et al., Bioconjug. Chem. 2004 May-Jun; 15 (3): 664-71; Nicaise et al., Protein Sci. 2004 Jul; 13 (7): 1882-91; Omidfar et al., Tumour Biol. 2004 Jul-Aug; 25 (4): 179-87; Omidfar et al., Tumour Biol. 2004 Sep-Dec; 25(5-6): 296-305; Szynol et al., Antimicrob Agents Chemother. 2004 Sep;48(9):3390-5; Saerens et al., J. Biol. Chem. 2004 Dec 10; 279 (50): 51965-72; De Genst et al., J. Biol. Chem. 2004 Dec 17; 279 (51): 53593-601; Dolk et al., Appl. Environ. Microbiol. 2005 Jan; 71(1): 442-50; Joosten et al., Appl Microbiol Biotechnol. 2005 Jan; 66(4): 384-92; Dumoulin et al., J. Mol. Biol. 2005 Feb 25; 346 (3): 773-88; Yau et al., J Immunol Methods. 2005 Feb; 297 (1-2): 213-24; De Genst et al., J. Biol. Chem. 2005 Apr 8; 280 (14): 14114-21; Huang et al., Eur. J. Hum. Genet. 2005 Apr 13; Dolk et al., Proteins. 2005 May 15; 59 (3): 555-64; Bond et al., J.

Mol. Biol. 2005 May 6;348(3):699-709; Zarebski et al., J. Mol. Biol. 2005 Apr 21; [E-publication ahead of print].

In accordance with the terminology used in the above references, the variable domains present in naturally occurring heavy chain antibodies will also be referred to as "*V_{HH} domains*", in order to distinguish them from the heavy chain variable domains that are present in conventional 4-chain antibodies (which will be referred to hereinbelow as "*V_H domains*") and from the light chain variable domains that are present in conventional 4-chain antibodies (which will be referred to hereinbelow as "*V_L domains*").

As mentioned in the prior art referred to above, V_{HH} domains have a number of unique structural characteristics and functional properties which make isolated V_{HH} domains (as well as Nanobodies based thereon, which share these structural characteristics and functional properties with the naturally occurring V_{HH} domains) and proteins containing the same highly advantageous for use as functional antigen-binding domains or proteins. In particular, and without being limited thereto, V_{HH} domains (which have been "designed" by nature to functionally bind to an antigen without the presence of, and without any interaction with, a light chain variable domain) and Nanobodies can function as a single, relatively small, functional antigen-binding structural unit, domain or protein. This distinguishes the V_{HH} domains from the V_H and V_L domains of conventional 4-chain antibodies, which by themselves are generally not suited for practical application as single antigen-binding proteins or domains, but need to be combined in some form or another to provide a functional antigen-binding unit (as in for example conventional antibody fragments such as Fab fragments; in ScFv's fragments, which consist of a V_H domain covalently linked to a V_L domain).

Because of these unique properties, the use of V_{HH} domains and Nanobodies as single antigen-binding proteins or as antigen-binding domains (i.e. as part of a larger protein or polypeptide) offers a number of significant advantages over the use of conventional V_H and V_L domains, scFv's or conventional antibody fragments (such as Fab- or F(ab')$_2$-fragments):

- only a single domain is required to bind an antigen with high affinity and with high selectivity, so that there is no need to have two separate domains present, nor to assure that these two domains are present in the right spacial conformation and configuration (i.e. through the use of especially designed linkers, as with scFv's);

- V_{HH} domains and Nanobodies can be expressed from a single gene and require no post-translational folding or modifications;
- V_{HH} domains and Nanobodies can easily be engineered into multivalent and multispecific formats (as further discussed herein);
- V_{HH} domains and Nanobodies are highly soluble and do not have a tendency to aggregate (as with the mouse-derived antigen-binding domains described by Ward et al., Nature, Vol. 341, 1989, p. 544);
- V_{HH} domains and Nanobodies are highly stable to heat, pH, proteases and other denaturing agents or conditions (see for example Ewert et al, supra);
- V_{HH} domains and Nanobodies are easy and relatively cheap to prepare, even on a scale required for production. For example, V_{HH} domains, Nanobodies and proteins/polypeptides containing the same can be produced using microbial fermentation (e.g. as further described below) and do not require the use of mammalian expression systems, as with for example conventional antibody fragments;
- V_{HH} domains and Nanobodies are relatively small (approximately 15 kDa, or 10 times smaller than a conventional IgG) compared to conventional 4-chain antibodies and antigen-binding fragments thereof, and therefore show high(er) penetration into tissues (including but not limited to solid tumors and other dense tissues) than such conventional 4-chain antibodies and antigen-binding fragments thereof;
- V_{HH} domains and Nanobodies can show so-called cavity-binding properties (inter alia due to their extended CDR3 loop, compared to conventional V_H domains) and can therefore also access targets and epitopes not accessable to conventional 4-chain antibodies and antigen-binding fragments thereof. For example, it has been shown that V_{HH} domains and Nanobodies can inhibit enzymes (see for example WO 97/49805; Transue et al., (1998), supra; Lauwereys et al., (1998), supra.

As mentioned above, the invention generally relates to Nanobodies directed against TNF-alpha, as well as to polypeptides comprising or essentially consisting of one or more of such Nanobodies, that can be used for the prophylactic, therapeutic and/or diagnostic purposes described below and in WO 04/041862.

As also mentioned above and further described below, the invention further relates to nucleic acids encoding such Nanobodies and polypeptides, to methods for preparing such Nanobodies and polypeptides, to host cells expressing or capable of expressing such

Nanobodies or polypeptides, to uses of such Nanobodies, polypeptides, nucleic acids or host cells, and to compositions comprising such Nanobodies, polypeptides, nucleic acids or host cells.

Generally, it should be noted that the term Nanobody as used herein in its broadest sense is not limited to a specific biological source or to a specific method of preparation. For example, as will be discussed in more detail below, the Nanobodies of the invention can be obtained (1) by isolating the V_{HH} domain of a naturally occurring heavy chain antibody; (2) by expression of a nucleotide sequence encoding a naturally occurring V_{HH} domain; (3) by "humanization" (as described below) of a naturally occurring V_{HH} domain or by expression of a nucleic acid encoding a such humanized V_{HH} domain; (4) by "camelization" (as described below) of a naturally occurring V_H domain from any animal species, in particular a species of mammal, such as from a human being, or by expression of a nucleic acid encoding such a camelized V_H domain; (5) by "camelisation" of a "domain antibody" or "Dab" as described by Ward et al (supra), or by expression of a nucleic acid encoding such a camelized V_H domain; (6) using synthetic or semi-synthetic techniques for preparing proteins, polypeptides or other amino acid sequences; (7) by preparing a nucleic acid encoding a Nanobody using techniques for nucleic acid synthesis, followed by expression of the nucleic acid thus obtained; and/or (8) by any combination of the foregoing. Suitable methods and techniques for performing the foregoing will be clear to the skilled person based on the disclosure herein and for example include the methods and techniques described in more detail hereinbelow.

However, according to a specific embodiment, the Nanobodies of the invention do not have an amino acid sequence that is exactly the same as (i.e. as a degree of sequence identity of 100% with) the amino acid sequence of a naturally occurring V_H domain, such as the amino acid sequence of a naturally occurring V_H domain from a mammal, and in particular from a human being.

One particularly preferred class of Nanobodies of the invention comprises Nanobodies with an amino acid sequence that corresponds to the amino acid sequence of a naturally occurring V_{HH} domain, but that has been "humanized" , i.e. by replacing one or more amino acid residues in the amino acid sequence of said naturally occurring V_{HH} sequence by one or more of the amino acid residues that occur at the corresponding position(s) in a V_H domain from a conventional 4-chain antibody from a human being (e.g. indicated above). This can be performed in a manner known per se, which will be clear to

the skilled person, for example on the basis of the further description below and the prior art on humanization referred to herein. Again, it should be noted that such humanized Nanobodies of the invention can be obtained in any suitable manner known per se (i.e. as indicated under points (1) – (8) above) and thus are not strictly limited to polypeptides that have been obtained using a polypeptide that comprises a naturally occurring V_{HH} domain as a starting material.

A preferred, but non-limiting humanzing substitution for Nanobodies belonging to the 103 P,R,S-group and/or the GLEW-group (as defined herein) is 108Q to 108L.

Another particularly preferred class of Nanobodies of the invention comprises Nanobodies with an amino acid sequence that corresponds to the amino acid sequence of a naturally occurring V_H domain that has been "camelized", i.e. by replacing one or more amino acid residues in the amino acid sequence of a naturally occurring V_H domain from a conventional 4-chain antibody by one or more of the amino acid residues that occur at the corresponding position(s) in a V_{HH} domain of a heavy chain antibody. This can be performed in a manner known per se, which will be clear to the skilled person, for example on the basis of the further description below. Reference is also made to WO 94/04678. Such camelization may preferentially occur at amino acid positions which are present at the V_H-V_L interface and at the so-called Camelidae hallmark residues (see for example also WO 94/04678), as also mentioned below. Preferably, the V_H domain or sequence that is used as a starting material or starting point for generating or designing the camelized Nanobody is preferably a V_H sequence from a mammal, more preferably the V_H sequence of a human being. However, it should be noted that such camelized Nanobodies of the invention can be obtained in any suitable manner known per se (i.e. as indicated under points (1) – (8) above) and thus are not strictly limited to polypeptides that have been obtained using a polypeptide that comprises a naturally occurring V_H domain as a starting material.

For example, again as further described below, both "humanization" and "camelization" can be performed by providing a nucleotide sequence that encodes such a naturally occurring V_{HH} domain or V_H domain, respectively, and then changing, in a manner known per se, one or more codons in said nucleotide sequence such that the new nucleotide sequence encodes a humanized or camelized Nanobody of the invention, respectively, and then expressing the nucleotide sequence thus obtained in a manner known per se so as to provide the desired Nanobody of the invention. Alternatively, based

on the amino acid sequence of a naturally occurring V_{HH} domain or V_H domain, respectively, the amino acid sequence of the desired humanized or camelized Nanobody of the invention, respectively, can be designed and then synthesized *de novo* using techniques for peptide synthesis known per se. Also, based on the amino acid sequence or nucleotide sequence of a naturally occurring V_{HH} domain or V_H domain, respectively, a nucleotide sequence encoding the desired humanized or camelized Nanobody of the invention, respectively, can be designed and then synthesized *de novo* using techniques for nucleic acid synthesis known per se, after which the nucleotide sequence thus obtained can be expressed in a manner known per se so as to provide the desired Nanobody of the invention.

Other suitable ways and techniques for obtaining Nanobodies of the invention and/or nucleotide sequences and/or nucleic acids encoding the same, starting from (the amino acid sequence of) naturally occurring V_H domains or preferably V_{HH} domains and/or from nucleotide sequences and/or nucleic acid sequences encoding the same will be clear from the skilled person, and may for example comprising combining one or more amino acid sequences and/or nucleotide sequences from naturally occurring V_H domains (such as one or more FR's and/or CDR's) with one or more one or more amino acid sequences and/or nucleotide sequences from naturally occurring V_{HH} domains (such an one or more FR's or CDR's), in a suitable manner so as to provide (a nucleotide sequence or nucleic acid encoding) a Nanobody of the invention.

According to one preferred, but non-limiting aspect of the aspect of the invention, a Nanobody in its broadest sense can be generally defined as a polypeptide comprising:

a) an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which the amino acid residue at position 108 according to the Kabat numbering is Q;

and/or:

b) an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which the amino acid residue at position 44 according to the Kabat numbering is E and in which the amino acid residue at position 45 according to the Kabat numbering is an R;

and/or:

113

c) an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of P, R and S, and is in particular chosen from the group consisting of R and S.

Thus, in a first preferred, but non-limiting aspect, a Nanobody of the invention may have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which

i) the amino acid residue at position 108 according to the Kabat numbering is Q;

and/or in which:

ii) the amino acid residue at position 44 according to the Kabat numbering is E and in which the amino acid residue at position 45 according to the Kabat numbering is an R;

and/or in which:

iii) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of P, R and S, and is in particular chosen from the group consisting of R and S;

and in which

iv) CDR1, CDR2 and CDR3 are as defined above, and are preferably as defined according to one of the preferred definitions above, and are more preferably as defined according to one of the more preferred definitions above.

In particular, a Nanobody against TNF-alpha according to the invention may have the structure:

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which

i) the amino acid residue at position 108 according to the Kabat numbering is Q;

and/or in which:

ii) the amino acid residue at position 44 according to the Kabat numbering is E and in which the amino acid residue at position 45 according to the Kabat numbering is an R;

and/or in which:

iii) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of P, R and S, and is in particular chosen from the group consisting of R and S;

and in which

iv) CDR1, CDR2 and CDR3 are as defined above, and are preferably as defined according to one of the preferred definitions above, and are more preferably as defined according to one of the more preferred definitions above.

In particular, according to one preferred, but non-limiting aspect of the aspect of the invention, a Nanobody can generally be defined as a polypeptide comprising an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which;

a-1) the amino acid residue at position 44 according to the Kabat numbering is chosen from the group consisting of G, E, D, G, Q, R, S, L; and is preferably chosen from the group consisting of G, E or Q; and

a-2) the amino acid residue at position 45 according to the Kabat numbering is chosen from the group consisting of L, R or C; and is preferably chosen from the group consisting of L or R; and

a-3) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of W, R or S; and is preferably W or R, and is most preferably W;

a-4) the amino acid residue at position 108 according to the Kabat numbering is Q;

or in which:

b-1) the amino acid residue at position 44 according to the Kabat numbering is chosen from the group consisting of E and Q; and

b-2) the amino acid residue at position 45 according to the Kabat numbering is R; and

b-3) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of W, R and S; and is preferably W;

b-4) the amino acid residue at position 108 according to the Kabat numbering is chosen from the group consisting of Q and L; and is preferably Q;

or in which:

c-1) the amino acid residue at position 44 according to the Kabat numbering is chosen from the group consisting of G, E, D, Q, R, S and L; and is preferably chosen from the group consisting of G, E and Q; and

c-2) the amino acid residue at position 45 according to the Kabat numbering is chosen from the group consisting of L, R and C; and is preferably chosen from the group consisting of L and R; and

c-3) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of P, R and S; and is in particular chosen from the group consisting of R and S; and

c-4) the amino acid residue at position 108 according to the Kabat numbering is chosen from the group consisting of Q and L; is preferably Q.

Thus, in another preferred, but non-limiting aspect, a Nanobody of the invention may have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

i) the amino acid residue at position 44 according to the Kabat numbering is chosen from the group consisting of G, E, D, G, Q, R, S, L; and is preferably chosen from the group consisting of G, E or Q;

and in which:

ii) the amino acid residue at position 45 according to the Kabat numbering is chosen from the group consisting of L, R or C; and is preferably chosen from the group consisting of L or R;

and in which:

iii) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of W, R or S; and is preferably W or R, and is most preferably W;

and in which

iv) the amino acid residue at position 108 according to the Kabat numbering is Q;

and in which:

v) CDR1, CDR2 and CDR3 are as defined above, and are preferably as defined according to one of the preferred definitions above, and are more preferably as defined according to one of the more preferred definitions above.

In another preferred, but non-limiting aspect, a Nanobody of the invention may have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

i) the amino acid residue at position 44 according to the Kabat numbering is chosen from the group consisting of E and Q;

and in which:

ii) the amino acid residue at position 45 according to the Kabat numbering is R;

and in which:

iii) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of W, R and S; and is preferably W;

and in which:

iv) the amino acid residue at position 108 according to the Kabat numbering is Q;

and in which:

vi) CDR1, CDR2 and CDR3 are as defined above, and are preferably as defined according to one of the preferred definitions above, and are more preferably as defined according to one of the more preferred definitions above.

In another preferred, but non-limiting aspect, a Nanobody of the invention may have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

i) the amino acid residue at position 44 according to the Kabat numbering is chosen from the group consisting of G, E, D, Q, R, S and L; and is preferably chosen from the group consisting of G, E and Q;

and in which:

ii) the amino acid residue at position 45 according to the Kabat numbering is chosen from the group consisting of L, R and C; and is preferably chosen from the group consisting of L and R;

and in which:

iii) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of P, R and S; and is in particular chosen from the group consisting of R and S;

and in which:

iv) the amino acid residue at position 108 according to the Kabat numbering is chosen from the group consisting of Q and L; is preferably Q;

and in which:

v) CDR1, CDR2 and CDR3 are as defined above, and are preferably as defined according to one of the preferred definitions above, and are more preferably as defined according to one of the more preferred definitions above.

Two particularly preferred, but non-limiting groups of the Nanobodies of the invention are those according to a) above; according to I) to a-4) above; according to b)

above; according to b-1) to b-4) above; according to c) above; and/or according to c-1) to c-4) above, in which;

a) the amino acid residues at positions 44-47 according to the Kabat numbering form the sequence GLEW (or a GLEW-like sequence as defined below) and the amino acid residue at position 108 is Q;

or in which:

b) the amino acid residues at positions 43-46 according to the Kabat numbering form the sequence KERE or KQRE (or a KERE-like sequence) and the amino acid residue at position 108 is Q or L, and is preferably Q.

Thus, in another preferred, but non-limiting aspect, a Nanobody of the invention may have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

i) the amino acid residues at positions 44-47 according to the Kabat numbering form the sequence GLEW (or a GLEW-like sequence as defined below) and the amino acid residue at position 108 is Q;

and in which:

ii) CDR1, CDR2 and CDR3 are as defined above, and are preferably as defined according to one of the preferred definitions above, and are more preferably as defined according to one of the more preferred definitions above.

In another preferred, but non-limiting aspect, a Nanobody of the invention may have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

i) the amino acid residues at positions 43-46 according to the Kabat numbering form the sequence KERE or KQRE (or a KERE-like sequence) and the amino acid residue at position 108 is Q or L, and is preferably Q;

and in which:

ii) CDR1, CDR2 and CDR3 are as defined above, and are preferably as defined according to one of the preferred definitions above, and are more preferably as defined according to one of the more preferred definitions above.

In the Nanobodies of the invention in which the amino acid residues at positions 43-46 according to the Kabat numbering form the sequence KERE or KQRE, the amino acid residue at position 37 is most preferably F. In the Nanobodies of the invention in which the amino acid residues at positions 44-47 according to the Kabat numbering form the sequence GLEW, the amino acid residue at position 37 is chosen from the group consisting of Y, H, I, V or F, and is most preferably F.

Thus, without being limited hereto in any way, on the basis of the amino acid residues present on the positions mentioned above, the Nanobodies of the invention can generally be classified is on the basis of the following three groups:

a) The "*GLEW-group*": Nanobodies with the amino acid sequence GLEW at positions 44-47 according to the Kabat numbering and Q at position 108 according to the Kabat numbering. As further described herein, Nanobodies within this group usually have a V at position 37, and can have a W, P, R or S at position 103, and preferably have a W at position 103. The GLEW group also comprises some GLEW-like sequences such as those mentioned in Table 2 below;

b) The "*KERE-group*": Nanobodies with the amino acid sequence KERE or KQRE or at positions 43-46 according to the Kabat numbering and Q or L at position 108 according to the Kabat numbering. As further described herein, Nanobodies within this group usually have a F at position 37, an L or F at position 47; and can have a W, P, R or S at position 103, and preferably have a W at position 103;

c) The "*103 P, R, S-group*": Nanobodies with a P R or S at position 103. These Nanobodies can have either the amino acid sequence GLEW at positions 44-47 of the Kabat numbering or the amino acid sequence KERE or KQRE at positions 43-46 according to the Kabat numbering, the latter most preferably in combination with an F at position 37 and an L or an F at position 47 (as defined for the KERE-

group); and can have Q or L at position 108 according to the Kabat numbering, and preferably have Q.

Thus, in another preferred, but non-limiting aspect, a Nanobody of the invention may be a Nanobody belonging to the GLEW-group (as defined herein), and in which CDR1, CDR2 and CDR3 are as defined above, and are preferably as defined according to one of the preferred definitions above, and are more preferably as defined according to one of the more preferred definitions above.

In another preferred, but non-limiting aspect, a Nanobody of the invention may be a Nanobody belonging to the KERE-group (as defined herein), and in which CDR1, CDR2 and CDR3 are as defined above, and are preferably as defined according to one of the preferred definitions above, and are more preferably as defined according to one of the more preferred definitions above.

Thus, in another preferred, but non-limiting aspect, a Nanobody of the invention may be a Nanobody belonging to the 103 P, R, S-group (as defined herein), and in which CDR1, CDR2 and CDR3 are as defined above, and are preferably as defined according to one of the preferred definitions above, and are more preferably as defined according to one of the more preferred definitions above.

Also, more generally and in addition to the 108Q, 43E/44R and 103P,R,S residues mentioned above, the Nanobodies of the invention can contain, at one or more positions that, in a conventional V_H domain, would form (part of) the V_H/V_L interface, contain one or more amino acid residues that are more highly charged than the amino acid residues that naturally occur at the same position(s) in the corresponding naturally occurring V_H or V_{HH} domain, and in particular one or more charged amino acid residues (as mentioned in Table 1).

Such substitutions include, but are not limited to the GLEW-like sequences mentioned in Table 2 below; as well as the substitutions that are described in the International Application WO 00/29004 for so-called "*microbodies*", e.g. a Q at position 108 and KLEW at positions 44-47.

In some embodiments of the Nanobodies of the invention, the amino acid residue at position 83 is chosen from the group consisting of L, M, S, V and W; and is preferably L.

Also, in some embodiments of the Nanobodies of the invention, the amino acid residue at position 83 is chosen from the group consisting of R, K, N, E, I and Q; and is most preferably either K or E (for Nanobodies corresponding to naturally occurring V_{HH}

domains) or R (for "humanized" Nanobodies, as described below). The amino acid residue at position 84 in some embodiments is chosen from the group consisting of P, A, R, S, D and V, and is most preferably P (for Nanobodies corresponding to naturally occurring V_{HH} domains) or R (for "humanized" Nanobodies, as described below).

Furthermore, in some embodiments of the Nanobodies of the invention, the amino acid residue at position 104 is chosen from the group consisting of G and D; and is most preferably G.

Collectively, the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108, which in the Nanobodies are as mentioned above, will also be referred to herein as the "*Hallmark Residues*". The Hallmark Residues and the amino acid residues at the corresponding positions of the most closely related human VH domain, VH3, are summarized in Table 2.

Some especially preferred combinations of these Hallmark Residues as occur in naturally occurring V_{HH} domains are mentioned in Table 3. For comparison, the corresponding amino acid residues of the human V_H3 called DP-47 have been indicated in italics.

Table 2: Hallmark Residues in Nanobodies

Position	Human V_H3	Hallmark Residues
11	L, V; predominantly L	L, M, S, V,W; preferably L
37	V, I, F; usually V	$F^{(1)}$, Y, H, I or V, preferably $F^{(1)}$ or Y
$44^{(8)}$	G	$G^{(2)}$, $E^{(3)}$, D, Q, R, S, L; preferably $G^{(2)}$, $E^{(3)}$ or Q; most preferably $G^{(2)}$ or $E^{(3)}$
$45^{(8)}$	L	$L^{(2)}$, $R^{(3)}$, C, I, L, P, Q, V; preferably $L^{(2)}$ or $R^{(3)}$
$47^{(8)}$	W, Y	$W^{(2)}$, $L^{(1)}$ or $F^{(1)}$, A, G, I, M, R, S or Y; preferably $W^{(2)}$, $L^{(1)}$, $F^{(1)}$ or R
83	R or K; usually R	R, $K^{(5)}$, N, $E^{(5)}$, I, M or Q; preferably K or R; most preferably K
84	A, T, D; predominantly A	$P^{(5)}$, A, L, R, S, D, V; preferably P

103	W	$W^{(4)}$, $P^{(6)}$, $R^{(6)}$, S; preferably W
104	G	G or D; preferably G
108	L, M or T; predominantly L	Q, $L^{(7)}$ or R; preferably Q or $L^{(7)}$

Notes:

(1): In particular, but not exclusively, in combination with KERE or KQRE at positions 43-46.

(2): Usually as GLEW at positions 44-47.

(3): Usually as KERE or KQRE at positions 43-46, e.g. as KEREL, KEREF, KQREL, KQREF or KEREG at positions 43-47. Alternatively, also sequences such as TERE (for example TEREL), KECE (for example KECEL or KECER), RERE (for example REREG), QERE (for example QEREG), KGRE (for example KGREG), KDRE (for example KDREV) are possible. Some other possible, but less preferred sequences include for example DECKL and NVCEL.

(4): With both GLEW at positions 44-47 and KERE or KQRE at positions 43-46.

(5): Often as KP or EP at positions 83-84 of naturally occurring V_{HH} domains.

(6): In particular, but not exclusively, in combination with GLEW at positions 44-47.

(7): With the proviso that when positions 44-47 are GLEW, position 108 is always Q.

(8): The GLEW group also contains GLEW-like sequences at positions 44-47, such as for example GVEW, EPEW, GLER, DQEW, DLEW, GIEW, ELEW, GPEW, EWLP, GPER, GLER and ELEW.

Table 3: Some preferred combinations of Hallmark Residues in naturally occurring Nanobodies.

For humanization of these combinations, reference is made to the specification.

	11	**37**	**44**	**45**	**47**	**83**	**84**	**103**	**104**	**108**
DP-47 (human)	*M*	*V*	*G*	*L*	*W*	*R*	*A*	*W*	*G*	*L*
"KERE" group	L	F	E	R	L	K	P	W	G	Q
	L	F	E	R	F	E	P	W	G	Q
	L	F	E	R	F	K	P	W	G	Q
	L	Y	Q	R	L	K	P	W	G	Q
	L	F	L	R	V	K	P	Q	G	Q
	L	F	Q	R	L	K	P	W	G	Q
	L	F	E	R	F	K	P	W	G	Q
"GLEW" group	L	V	G	L	W	K	S	W	G	Q
	M	V	G	L	W	K	P	R	G	Q

In the Nanobodies, each amino acid residue at any other position than the Hallmark Residues can be any amino acid residue that naturally occurs at the corresponding position (according to the Kabat numbering) of a naturally occurring V_{HH} domain.

Such amino acid residues will be clear to the skilled person. Tables 4 - 7 mention some non-limiting residues that can be present at each position (according to the Kabat numbering) of the FR1, FR2, FR3 and FR4 of naturally occurring V_{HH} domains. For each position, the amino acid residue that most frequently occurs at each position of a naturally occurring V_{HH} domain (and which is the most preferred amino acid residue for said position in a Nanobody) is indicated in bold; and other preferred amino acid residues for each position have been underlined (note: the number of amino acid residues that are found at positions 26-30 of naturally occurring V_{HH} domains supports the hypothesis underlying the numbering Chothia (supra) that the residues at these positions already form part of CDR1.)

In Tables 4 - 7, some of the non-limiting residues that can be present at each position of a human V_H3 domain have also been mentioned. Again, for each position, the amino acid residue that most frequently occurs at each position of a naturally occurring human V_H3 domain is indicated in bold; and other preferred amino acid residues have been underlined.

Table 4: Non-limiting examples of amino acid residues in FR1 (for the footnotes, see the footnotes to Table 2)

Pos.	Amino acid residue(s):	
	Human V_H3	*Camelid V_{HH}'s*
1	**E**, Q	**Q**, A, E, D, H, R
2	**V**	**V**, A, E, G, L, M, Q
3	**Q**	**Q**, K, E, H, P, R, Y
4	**L**	**L**, F, P, R, V
5	**V**, L	**Q**, E, L, V, M, P, A, I
6	**E**	**E**, D, Q, A, H
7	**S**, T	**S**, F, H
8	**G**, R	**G**, A, R
9	**G**	**G**, E
10	**G**, V	**G**, D, R, A, E, N, T, V
11	**Hallmark residue:** L, M, S, V,W, F, N, P, T, Y; preferably L	
12	**V**, I	**V**, A, G, M
13	**Q**, K, R	**Q**, E, K, D, G, A, H, L, N, P, R, T
14	**P**	**A**, Q, A, G, P, T, V, E, F, I, N, S
15	**G**	**G**
16	**G**, R	**G**, A, E, D, N, P, R, S, V, W
17	**S**	**S**, F, T, N, P, A, C
18	**L**	**L**, V, M, Q, R
19	**R**, K	**R**, K, L, N, S, T, A, F, G, I, M, Q
20	**L**	**L**, F, I, V, M, S
21	**S**	**S**, F, T, G, H, P, A
22	**C**	**C**
23	**A**, T	**A**, D, P, S, T, V, E, G, I, L, Q, R
24	**A**	**A**, I, S, T, V, C, E, F, G, L, N, P, Q, Y

Table 4: Non-limiting examples of amino acid residues in FR1 (continued)

Pos.	Amino acid residue(s):	
	Human V_H3	*Camelid V_{HH}'s*
25	**S**	**S**, A, F, P, T, L, V
26	**G**	**G**, D, E, R, S, V, A, I, M, P, T
27	**F**	S, F, R, L, P, G, N, A, D, E, H, I, K, M, Q, T, V, Y
28	**T**	N, T, E, D, S, I, R, A, G, R, F, Y, L, M, P, V
29	**F**, V	F,L, D, S, I, G, V, A, E, P, T, Y
30	**S**, D, G	N, S, E, G, A, D, M, T, H, I, P, R, V, W

Table 5: Non-limiting examples of amino acid residues in FR2 (for the footnotes, see the footnotes to Table 2)

Pos.	Amino acid residue(s):	
	Human V_H3	*Camelid V_{HH}'s*
36	**W**	**W**
37	**Hallmark residue:** $F^{(1)}$, Y, H, I, A, L, P, S or V preferably $F^{(1)}$ or Y	
38	**R**	**R**
39	**Q**	**Q**, H, P, R, A, D, G, L, E
40	**A**	**A**, F, G, P, T, V, I, L, N, R, S, Y
41	**P**, S, T	**P**, A, L, S, I, Q, T
42	**G**	**G**, E, D, R, T, V
43	**K**	**K**, D, E, N, Q, R, T, V, A, L, M, S
44	**Hallmark residue:** $G^{(2)}$, $E^{(3)}$, D, Q, R, S, L, A, F, K, M, N, P, V, W, Y; preferably $G^{(2)}$, $E^{(3)}$ or Q; most preferably $G^{(2)}$ or $E^{(3)}$	
45	**Hallmark residue**: $L^{(2)}$, $R^{(3)}$, C, I, L, P, Q, V, D, E, G, H, K, T; preferably $L^{(2)}$ or $R^{(3)}$	

46	**E**, V	**E**, D, K, Q, V, A, G, N
47	**Hallmark residue:** $W^{(2)}$, $L^{(1)}$ or $F^{(1)}$, A, G, I, M, R, S, D, E, H, K, Q, T, V or Y; preferably $W^{(2)}$, $L^{(1)}$, $F^{(1)}$ or R	
48	**V**	**V**, I, L, A, C, E, F, G, H, M, P, Q, R, S, T, V, W, Y
49	**S**, A, G	**A**, S, A, G, T, V, D, E, I, L, Q, R, Y

Table 6: Non-limiting examples of amino acid residues in FR3 (for the footnotes, see the footnotes to Table 2)

Pos.	**Amino acid residue(s):**	
	Human V_H3	*Camelid V_{HH}'s*
66	**R**	**R**
67	**F**	**F**, L, V, A, D, I, S, Y
68	**T**	**T**, A, S, D, F, G, I, K, N
69	**I**	**I**, M, V, A, F, L, R, S, T
70	**S**	**S**, A, F, E, G, K, P, T, V
71	**R**	**R**, G, I, K, Q, S, T, W, A, F, L, M, N
72	**D**, E	**D**, E, G, N, V, A, H, I, L, Q, S, T
73	**N**, D, G	**N**, D, F, I, K, S, T, Y, A, G, H, L, M, R, V
74	**A**, S	**A**, D, G, N, P, S, T, F, H, I, L, R, V, Y
75	**K**	**K**, A, E, K, L, N, Q, R, D, G, I, M, S, T, V, W
76	**N**, S	**N**, D, K, R, S, T, Y, E, G, H, I, Q
77	S, T, I	**T**, A, E, I, M, S , K, L, N, R, V
78	**L**, A	**V**, L,A, F, G, I, M, E, N, Q, R, S, T, W
79	**Y**, H	**Y**, A, D, F, H, S, T, C, E, I, L, N, V, W

80	**L**	**L**, F, V, M
81	**Q**	**Q**, E, R, T, G, H, I, K, L, M, N
82	**M**	**M**, I, L, V, G, P, T
82a	**N**, G	**N**, D, G, H, S, T, A, E, I, K, R, V
82b	**S**	**S**, N, D, G, R, A, C, E, F, I, K, M, P, T, V
82c	**L**	**L**, P, M, T, V
83	**Hallmark residue:** R, K[5], N, E[5], I, M, A, D, G, L, Q, S, T or Q; preferably K or R; most preferably K	
84	**Hallmark residue:** P[5], A, L, R, S, D, V, F, G, H, N, T, Y; preferably P	
85	**E**, G	**E**, D, G, Q, A, N, R, V, Y
86	**D**	**D**, E, F, Y
87	**T**, M	**T**, S, A, C, M

Table 6: Non-limiting examples of amino acid residues in FR3 (continued)

Pos.	Amino acid residue(s):	
	Human V_H3	*Camelid V_{HH}'s*
88	**A**	**A**, G, S, D, L, N, P
89	**V**, L	**V**, A, D, I, L, M, N, R, T, E, F, S
90	**Y**	**Y**, F, E, H, N
91	**Y**, H	**Y**, D, F, H, L, S, T, V, C, I, N, R, W
92	**C**	**C**
93	**A**, K, T	**A**, N, G, H, K, R, S, T, V, Y, E, F, I, L, M, Q
94	**K**, R, T	**A**, V, C, F, G, I, L, R, S, D, E, K, M, N, P, Q, T, W, Y T or K;

Table 7: Non-limiting examples of amino acid residues in FR4 (for the footnotes, see the footnotes to Table 2)

Pos.	Amino acid residue(s):	
	Human V_H3	*Camelid V_{HH}'s*
103	**Hallmark residue:** W[4], P[6], R[6], S, F, G, K, L, N, Q, V, Y; preferably W	
104	**Hallmark residue:** G, A, R, S, T or D; preferably G	
105	**Q**, R	**Q**, E, K, P, R, G, H, L, S, V
106	**G**	**G**
107	**T**	**T**, A, I, N, P
108	**Hallmark residue:** Q, L[7], E, H, N, P, T or R; preferably Q or L[7]	
109	**V**	**V**
110	**T**	**T**, I, A
111	**V**	**V**, A, I, G
112	**S**	**S**, F, A, L, P, T, Y
113	**S**	**S**, A, L, P, F, T

Thus, in another preferred, but not limiting aspect, a Nanobody of the invention can have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

i) the Hallmark residues are as defined above;

and in which:

ii) CDR1, CDR2 and CDR3 are as defined above, and are preferably as defined according to one of the preferred definitions above, and are more preferably as defined according to one of the more preferred definitions above.

In another preferred, but not limiting aspect, a Nanobody of the invention can have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

and in which

i) FR1 is chosen from the group consisting of the amino acid sequence:

[1] QVQLQESGGGXVQAGGSLRLSCAASG [26] [SEQ ID NO: 1]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with the above amino acid sequence; in which

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 4; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 4; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and in which:

ii) FR2 is chosen from the group consisting of the amino acid sequence:

[36] W<u>X</u>RQAPGK<u>XX</u>E<u>X</u>VA [49] [SEQ ID NO: 2]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with the above amino acid sequence; in which

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 5; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 5; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and in which:

iii) FR3 is chosen from the group consisting of the amino acid sequence:

[66] RFTISRDNAKNTVYLQMNSL**XX**EDTAVYYCAA [94] [SEQ ID NO: 3]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with the above amino acid sequence; in which

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 6; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 6; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and in which:

iv) FR4 is chosen from the group consisting of the amino acid sequence:

[103] **XX**QGT**X**VTVSS [113] [SEQ ID NO: 4]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with the above amino acid sequence; in which

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 6; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 6; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and in which:

v) CDR1, CDR2 and CDR3 are as defined above, and are preferably as defined according to one of the preferred definitions above, and are more preferably as defined according to one of the more preferred definitions above;

in which the Hallmark Residues are indicated by "**X**" and are as defined hereinabove and in which the numbers between brackets refer to the amino acid positions according to the Kabat numbering.

In another preferred, but not limiting aspect, a Nanobody of the invention can have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

and in which

i) FR1 is chosen from the group consisting of the amino acid sequence:

[1] QVQLQESGGGLVQAGGSLRLSCAASG [26] [SEQ ID NO: 5]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with the above amino acid sequence; in which

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 4; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and

(3) the Hallmark residue at position is as indicated in the sequence above;

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 4; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and

(3) the Hallmark residue at position is as indicated in the sequence above;

and in which:

ii) FR2 is chosen from the group consisting of the amino acid sequences:

[36] W**F**RQAPGK**ER**E**L**VA [49] [SEQ ID NO: 6]

[36] W**F**RQAPGK**ER**E**F**VA [49] [SEQ ID NO: 7]

[36] W**F**RQAPGK**ER**E**G**A [49] [SEQ ID NO: 8]

[36] W**F**RQAPGK**QR**E**L**VA [49] [SEQ ID NO: 9]

[36] W**F**RQAPGK**QR**E**F**VA [49] [SEQ ID NO: 10]

[36] W**Y**RQAPGK**GL**E**W**A [49] [SEQ ID NO: 11]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the above amino acid sequences; in which

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 5; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and

(3) the Hallmark residues at positions 37, 44, 45 and 47 are as indicated in each of the sequences above;

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 5; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and

(3) the Hallmark residues at positions 37, 44, 45 and 47 are as indicated in each of the sequences above;

and in which:

iii) FR3 is chosen from the group consisting of the amino acid sequence:

[66] RFTISRDNAKNTVYLQMNSL**KP**EDTAVYYCAA [94] [SEQ ID NO: 12]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least

99% sequence identity (as defined herein) with the above amino acid sequence; in which

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 6; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and

(3) the Hallmark residues at positions 83 and 84 are as indicated in each of the sequences above;

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 6; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and

(3) the Hallmark residues at positions 83 and 84 are as indicated in each of the sequences above;

and in which:

iv) FR4 is chosen from the group consisting of the amino acid sequences:

[103] **WG**QGT**Q**VTVSS [113] [SEQ ID NO: 13]

[103] **WG**QGT**L**VTVSS [113] [SEQ ID NO: 14]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the above amino acid sequence; in which

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 6; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and

(3) the Hallmark residues at positions 103, 104 and 108 are as indicated in each of the sequences above;

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 6; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and

(3) the Hallmark residues at positions 103, 104 and 108 are as indicated in each of the sequences above;

and in which:

v) CDR1, CDR2 and CDR3 are as defined above, and are preferably as defined according to one of the preferred definitions above, and are more preferably as defined according to one of the more preferred definitions above.

In another preferred, but not limiting aspect, a Nanobody of the invention can have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which: and in which

i) FR1 is chosen from the group consisting of the amino acid sequence:

[1] QVQLQESGGG**L**VQAGGSLRLSCAASG [26] [SEQ ID NO: 5]

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 4; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and

(3) the Hallmark residue at position is as indicated in the sequence above;

and in which:

ii) FR2 is chosen from the group consisting of the amino acid sequences:

[36] W**F**RQAPGK**ER**E**L**VA [49]	[SEQ ID NO: 6]
[36] W**F**RQAPGK**ER**E**F**VA [49]	[SEQ ID NO: 7]
[36] W**F**RQAPGK**ER**E**G**A [49]	[SEQ ID NO: 8]
[36] W**F**RQAPGK**QR**E**L**VA [49]	[SEQ ID NO: 9]
[36] W**F**RQAPGK**QR**E**F**VA [49]	[SEQ ID NO: 10]

and/or from the group consisting of amino acid sequences that have 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 5; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and

(3) the Hallmark residues at positions 37, 44, 45 and 47 are as indicated in each of the sequences above;

and in which:

iii) FR3 is chosen from the group consisting of the amino acid sequence:

[66] RFTISRDNAKNTVYLQMNSL**KP**EDTAVYYCAA [94] [SEQ ID NO: 12]

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 6; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and

(3) the Hallmark residues at positions 83 and 84 are as indicated in each of the sequences above;

and in which:

iv) FR4 is chosen from the group consisting of the amino acid sequences:

[103] **WG**QGT**Q**VTVSS [113] [SEQ ID NO: 13]

[103] **WG**QGT**L**VTVSS [113] [SEQ ID NO: 14]

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 7; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and

(3) the Hallmark residues at positions 103, 104 and 108 are as indicated in each of the sequences above;

and in which:

v) CDR1, CDR2 and CDR3 are as defined above, and are preferably as defined according to one of the preferred definitions above, and are more preferably as defined according to one of the more preferred definitions above.

In another preferred, but not limiting aspect, a Nanobody of the invention can have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which: and in which

i) FR1 is chosen from the group consisting of the amino acid sequence:

[1] QVQLQESGGG**L**VQAGGSLRLSCAASG [26] [SEQ ID NO: 5]

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 4; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and

(3) the Hallmark residue at position is as indicated in the sequence above;

and in which:

ii) FR2 is chosen from the group consisting of the amino acid sequence:

[36] W**Y**RQAPGK**GL**E**W**A [49] [SEQ ID NO: 11]

and/or from the group consisting of amino acid sequences that have 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 5; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and

(3) the Hallmark residues at positions 37, 44, 45 and 47 are as indicated in each of the sequences above;

and in which:

iii) FR3 is chosen from the group consisting of the amino acid sequence:

[66] RFTISRDNAKNTVYLQMNSL**KP**EDTAVYYCAA [94] [SEQ ID NO: 12]

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 6; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and

(3) the Hallmark residues at positions 83 and 84 are as indicated in each of the sequences above;

and in which:

iv) FR4 is chosen from the group consisting of the amino acid sequence:

[103] **WG**QGT**Q**VTVSS [113] [SEQ ID NO: 13]

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 7; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and

(3) the Hallmark residues at positions 103, 104 and 108 are as indicated in each of the sequences above;

and in which:

v) CDR1, CDR2 and CDR3 are as defined above, and are preferably as defined according to one of the preferred definitions above, and are more preferably as defined according to one of the more preferred definitions above.

Some other framework sequences that can be present in the Nanobodies of the invention can be found in the European patent EP 656 946 mentioned above (see for example also the granted US equivalent 5,759,808).

In another preferred, but not limiting aspect, a Nanobody of the invention can have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which: and in which

i) FR1 is chosen from the group consisting of the FR1 sequences present in the Nanobodies of SEQ ID NO's 52 to 60, SEQ ID NO's 76 to 86 or SEQ ID NO's 95 to 99, and in particular in the humanized Nanobodies of SEQ ID NO's 76 to 86 or SEQ ID NO's 95 to 99,

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of said FR1 sequences; in which

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 4; and/or

143

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to said FR1 sequence; and

(3) the Hallmark residue at position is as indicated in said FR1 sequence;

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of said FR1 sequences, in which:

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 4; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to said FR1 sequence; and

(3) the Hallmark residue at position is as indicated in said FR1 sequence;

and in which:

ii) FR2 is chosen from the group consisting of the FR2 sequences present in the Nanobodies of SEQ ID NO's 52 to 60, SEQ ID NO's 76 to 86 or SEQ ID NO's 95 to 99, and in particular in the humanized Nanobodies of SEQ ID NO's 76 to 86 or SEQ ID NO's 95 to 99,

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of said FR2 sequences; in which

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 5; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to said FR2 sequence; and

(3) the Hallmark residues at positions 37, 44, 45 and 47 are as indicated in said FR2 sequence;

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of said FR2 sequences, in which:

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 5; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to said FR2 sequence; and

(3) the Hallmark residues at positions 37, 44, 45 and 47 are as indicated in said FR2 sequence;

and in which:

iii) FR3 is chosen from the group consisting of the FR3 sequences present in the Nanobodies of SEQ ID NO's 52 to 60, SEQ ID NO's 76 to 86 or SEQ ID NO's 95 to 99, and in particular in the humanized Nanobodies of SEQ ID NO's 76 to 86 or SEQ ID NO's 95 to 99,

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of said FR3 sequences; in which

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 6; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to said FR3 sequence; and

(3) the Hallmark residues at positions 83 and 84 are as indicated in said FR3 sequence;

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of said FR3 sequences, in which:

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 6; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to said FR3 sequence; and

(3) the Hallmark residues at positions 83 and 84 are as indicated in said FR3 sequence;

and in which:

iv) FR4 is chosen from the group consisting of the FR4 sequences present in the Nanobodies of SEQ ID NO's 52 to 60, SEQ ID NO's 76 to 86 or SEQ ID NO's 95 to

99, and in particular in the humanized Nanobodies of SEQ ID NO's 76 to 86 or SEQ ID NO's 95 to 99,

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of said FR4 sequences; in which

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 6; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to said FR4 sequence; and

(3) the Hallmark residues at positions 103, 104 and 108 are as indicated in said FR3 sequence;

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of said FR4 sequences, in which:

(1) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table 6; and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to said FR4 sequence; and

(3) the Hallmark residues at positions 103, 104 and 108 are as indicated in said FR4 sequence;

and in which:

v) CDR1, CDR2 and CDR3 are as defined above, and are preferably as defined according to one of the preferred definitions above, and are more preferably as defined according to one of the more preferred definitions above.

Some particularly preferred Nanobodies of the invention can be chosen from the group consisting of the amino acid sequences of SEQ ID NO's 52 to 60, SEQ ID NO's 76 to 86 or SEQ ID NO's 95 to 99, and in particular in the humanized Nanobodies of SEQ ID NO's 76 to 86 or SEQ ID NO's 95 to 99 or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the amino acid

sequences of SEQ ID NO's 52 to 60, SEQ ID NO's 76 to 86 or SEQ ID NO's 95 to 99; in which

(1) the Hallmark residues can be as indicated in Table 2 above;

(2) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Tables 4-7; and/or

(3) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s).

Some even more particularly preferred Nanobodies of the invention can be chosen from the group consisting of the amino acid sequences of SEQ ID NO's 52 to 60, SEQ ID NO's 76 to 86 or SEQ ID NO's 95 to 99, and in particular in the humanized Nanobodies of SEQ ID NO's 76 to 86 or SEQ ID NO's 95 to 99 or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the amino acid sequences of SEQ ID NO's 52 to 60, SEQ ID NO's 76 to 86 or SEQ ID NO's 95 to 99; in which

(1) the Hallmark residues are as indicated in the pertinent sequence chosen from SEQ ID NO's 52 to 60, SEQ ID NO's 76 to 86 or SEQ ID NO's 95 to 99;

(2) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Tables 4-7; and/or

(3) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the pertinent sequence chosen from SEQ ID NO's 52 to 60, SEQ ID NO's 76 to 86 or SEQ ID NO's 95 to 99.

Some of the most preferred Nanobodies of the invention can be chosen from the group consisting of the amino acid sequences of SEQ ID NO's 52 to 60, SEQ ID NO's 76 to 86 or SEQ ID NO's 95 to 99, and in particular from the humanized Nanobodies of SEQ ID NO's 76 to 86 or SEQ ID NO's 95 to 99.

As will be clear from the above, the term Nanobodies of the invention as used herein in its broadest sense also comprises natural or synthetic mutants, variants, alleles, analogs and

orthologs (hereinbelow collectively referred to as "*analogs*") of the Nanobodies mentioned in the SEQ ID NO's 52 to 60, SEQ ID NO's 76 to 86 or SEQ ID NO's 95 to 99.

Generally, such analogs can for example comprise homologous sequences, functional portions, or a functional portion of a homologous sequence (as further defined below) of a Nanobody. Generally, in such analogs, each amino acid residue (other than the Hallmark Residue) in each of the framework regions can be replaced by any other amino acid residue, provided that the total degree of sequence identity of the framework regions remains as defined above. Preferably, however, in such analogs:

- one or amino acid residues in the above framework sequences are replaced by one or more amino acid residues that naturally occur at the same position in a naturally occurring V_{HH} domain. Some examples of such substitutions are mentioned in Tables 4-7 above;

and/or:

- one or amino acid residues in the above framework sequences are replaced by one or more amino acid residues that can be considered a "conservative" amino acid substitution, as described hereinabove;

and/or:

- one or amino acid residues in the above framework sequences are replaced by one or more amino acid residues that naturally occur at the same position in a naturally occurring V_H domain of a human being. This is generally referred to as "humanization" of the naturally occurring V_{HH}/Nanobody in general and of said position in particular, and will be discussed in more detail hereinbelow;

and:

- positions for which only one amino acid residue is mentioned for both the V_H domain and the V_{HH} domain in Tables 4 – 7 above are preferably not replaced.

Also, although generally less preferred, in such analogs, one or more amino acid residues may be deleted from the framework regions and/or inserted into the framework regions (optionally in addition to one or more amino acid substitutions as mentioned above), provided that the total degree of sequence identity of the framework regions remains as defined above. The Hallmark residues should not be deleted. Also, most preferably, amino acid residues for which only one amino acid residue is mentioned for both the V_H domain and the V_{HH} domain in Tables 4 – 7 above are preferably not deleted.

Preferably, such analogs should be such that they still can bind to, have affinity for and/or have specificity for TNF-alpha, i.e. with an affinity and/or a specificity which is at least 10%, preferably at least 50%, more preferably at least 70%, even more preferably at least 80%, such as at least 90%, at least 95%, at least 99% or more, of the affinity and/or specificity of at least one of the Nanobodies of SEQ ID No's SEQ ID NO's 52 to 60, SEQ ID NO's 76 to 86 or SEQ ID NO's 95 to 99, as determined using a suitable assay, for example an assay to determine binding of the analog to TNF, and in particular one of the assays as used in the Examples below.

Generally, such analogs can for example be obtained by providing a nucleic acid that encodes a naturally occurring V_{HH} domain, changing the codons for the one or more amino acid residues that are to be humanized into the codons for the corresponding human amino acid residue(s), expressing the nucleic acid/nucleotide sequence thus obtained in a suitable host or expression system; and optionally isolating and/or purifying the analog thus obtained to provide said analog in essentially isolated form (as defined hereinabove). This can generally be performed using methods and techniques known per se, which will be clear to the skilled person, for example from the handbooks and references cited herein and/or from the further description hereinbelow. Alternatively, and for example, a nucleic acid encoding an analog can be synthesized in a manner known per se (for example using an automated apparatus for synthesizing nucleic acid sequences with a predefined amino acid sequence) and can be expressed in a suitable host or expression system, upon which the analog thus obtained can optionally be isolated and/or purified so as to provide said analog in essentially isolated form (as defined hereinabove). Another way to provide the analogs involves chemical synthesis of the pertinent amino acid sequence using techniques for peptide synthesis known per se, such as those mentioned hereinbelow.

It will be also generally be clear to the skilled person that Nanobodies (including analogs thereof) can also be prepared starting from human V_H sequences (i.e. amino acid sequences or the corresponding nucleotide sequences), such as for example human V_H3 sequences such as DP-47, DP-51, DP-54 or DP-29, by changing one or more amino acid residues in the amino acid sequence of said human V_H domain, so as to provide an amino acid sequence that has (a) a Q at position 108; and/or (b) E at position 44 and/or R at position 45, and preferably E at position 44 and R at position 45; and/or (c) P, R or S at position 103, as described above. Again, this can generally be performed using the various methods and

techniques referred to in the previous paragraph, using an amino acid sequence and/or nucleotide sequence for a human V_H domain as a starting point.

The term Nanobodies as used herein in its broadest sense also comprises parts or fragments of the Nanobodies (including analogs) of the invention as defined above, which can again be as further described below.

Generally, parts or fragments of the Nanobodies and/or analogs have amino acid sequences in which, compared to the amino acid sequence of the corresponding full length Nanobody or analog, one or more of the amino acid residues at the N-terminal end, one or more amino acid residues at the C-terminal end, one or more contiguous internal amino acid residues, or any combination thereof, have been deleted and/or removed. It is also possible to combine one or more of such parts or fragments to provide a Nanobody of the invention.

Preferably, the amino acid sequence of a Nanobody that comprises one or more parts or fragments of a full length Nanobody and/or analog should have a degree of sequence identity of at least 50%, preferably at least 60%, more preferably at least 70%, such as at least 80%, at least 90% or at least 95%, with the amino acid sequence of the corresponding full length Nanobody.

Also, the amino acid sequence of a Nanobody that comprises one or more parts or fragments of a full length Nanobody and/or analog is preferably such that is comprises at least 10 contiguous amino acid residues, preferably at least 20 contiguous amino acid residues, more preferably at least 30 contiguous amino acid residues, such as at least 40 contiguous amino acid residues, of the amino acid sequence of the corresponding full length Nanobody.

Generally, such parts or fragments of the Nanobodies of the invention will have amino acid sequences in which, compared to the amino acid sequence of the corresponding full length Nanobody of the invention, one or more of the amino acid residues at the N-terminal end, one or more amino acid residues at the C-terminal end, one or more contiguous internal amino acid residues, or any combination thereof, have been deleted and/or removed. It is also possible to combine one or more of such parts or fragments to provide a Nanobody of the invention.

According to one preferred embodiment, a fragment as used herein comprises at least one of the CDR's present in a full-sized Nanobody of the invention, preferably at least two of the CDR's present in a full-sized Nanobody of the invention, more preferably at least CDR2

and CDR3 present in a full-sized Nanobody of the invention, such as for example all three CDR's present in a full-sized Nanobody of the invention.

According to another particularly preferred, but non-limiting embodiment, such a part or fragment comprises at least FR3, CDR3 and FR4 of the corresponding full length Nanobody of the invention, i.e. as for example described in the International application WO 03/050531 (Lasters et al.).

Preferably, such parts or fragments should be such that they still can bind to, have affinity for and/or have specificity for TNF-alpha, i.e. with an affinity and/or a specificity which is at least 10%, preferably at least 50%, more preferably at least 70%, even more preferably at least 80%, such as at least 90%, at least 95%, at least 99% or more, of the affinity and/or specificity of the corresponding full-sized Nanobody of the invention, for example an assay to determine binding of the analog to TNF, and in particular one of the assays as used in the Examples below.

From the description hereinabove, it will be clear that the amino acid sequences of the Nanobodies used herein differ at at least one amino acid position in at least one of the framework regions from the amino acid sequences of naturally occurring V_H domains, such as the amino acid sequences of naturally occurring V_H domains of antibodies from human beings. In particular, it will be clear that the amino acid sequences of the Nanobodies used herein differ at at least one of the Hallmark Residues from amino acid sequences of naturally occurring V_H domains, such as the amino acid sequences of naturally occurring V_H domains from antibodies from Camelids and/or human beings.

Thus, according to one specific embodiment, a Nanobody of the invention has an amino acid sequence that differs at at least one amino acid position in one of the framework regions from the amino acid sequence of a naturally occurring V_H domain. According to a more specific, but non-limiting embodiment of the invention, a Nanobody of the invention has an amino acid sequence that differs at at least one of the Hallmark residues from the amino acid sequence of a naturally occurring V_H domain.

From the description hereinabove, it will also be clear that the amino acid sequences of the some of the Nanobodies of the invention, such as the humanized Nanobodies of the invention, will differ at at least one amino acid position in at least one of the framework regions (i.e. either at the position of a Hallmark residue or at another position) from the amino acid sequences of naturally occurring V_{HH} domains. Thus, according to one specific, but non-limiting embodiment, a Nanobody of the invention has an amino acid sequence that

differs at at least one amino acid position in one of the framework regions from the amino acid sequence of a naturally occurring V_{HH} domain. According to a more specific, but non-limiting embodiment of the invention, a Nanobody of the invention has an amino acid sequence that differs at at least one of the Hallmark residues from the amino acid sequence of a naturally occurring V_{HH} domain.

The invention in its broadest sense also comprises derivatives of the Nanobodies of the invention. Such derivatives can generally be obtained by modification, and in particular by chemical and/or biological (e.g enzymatical) modification, of the Nanobodies of the invention and/or of one or more of the amino acid residues that form the Nanobodies of the invention.

Examples of such modifications, as well as examples of amino acid residues within the Nanobody sequence that can be modified in such a manner (i.e. either on the protein backbone but preferably on a side chain), methods and techniques that can be used to introduce such modifications and the potential uses and advantages of such modifications will be clear to the skilled person.

For example, such a modification may involve the introduction (e.g. by covalent linking or in an other suitable manner) of one or more functional groups, residues or moieties into or onto the Nanobody of the invention, and in particular of one or more functional groups, residues or moieties that confer one or more desired properties or functionalities to the Nanobody of the invention. Example of such functional groups will be clear to the skilled person.

For example, such modification may comprise the introduction (e.g. by covalent binding or in any other suitable manner) of one or more functional groups that that increase the half-life, the solubility and/or the absorption of the Nanobody of the invention, that reduce the immunogenicity and/or the toxicity of the Nanobody of the invention, that eliminate or attenuate any undesirable side effects of the Nanobody of the invention, and/or that confer other advantageous properties to and/or reduce the undesired properties of the Nanobodies and/or polypeptides of the invention; or any combination of two or more of the foregoing. Examples of such functional groups and of techniques for introducing them will be clear to the skilled person, and can generally comprise all functional groups and techniques mentioned in the general background art cited hereinabove as well as the functional groups and techniques known per se for the modification of pharmaceutical proteins, and in particular for the modification of antibodies or antibody fragments (including ScFv's and

single domain antibodies), for which reference is for example made to Remington's Pharmaceutical Sciences, 16th ed., Mack Publishing Co., Easton, PA (1980). Such functional groups may for example be linked directly (for example covalently) to a Nanobody of the invention, or optionally via a suitable linker or spacer, as will again be clear to the skilled person.

One of the most widely used techniques for increasing the half-life and/or the reducing immunogenicity of pharmaceutical proteins comprises attachment of a suitable pharmacologically acceptable polymer, such as poly(ethyleneglycol) (PEG) or derivatives thereof (such as methoxypoly(ethyleneglycol) or mPEG). Generally, any suitable form of pegylation can be used, such as the pegylation used in the art for antibodies and antibody fragments (including but not limited to (single) domain antibodies and ScFv's); reference is made to for example Chapman, Nat. Biotechnol., 54, 531-545 (2002); by Veronese and Harris, Adv. Drug Deliv. Rev. 54, 453-456 (2003), by Harris and Chess, Nat. Rev. Drug. Discov., 2, (2003) and in WO 04/060965. Various reagents for pegylation of proteins are also commercially available, for example from Nektar Therapeutics, USA.

Preferably, site-directed pegylation is used, in particular via a cysteine-residue (see for example Yang et al., Protein Engineering, 16, 10, 761-770 (2003). For example, for this purpose, PEG may be attached to a cysteine residue that naturally occurs in a Nanobody of the invention, a Nanobody of the invention may be modified so as to suitably introduce one or more cysteine residues for attachment of PEG, or an amino acid sequence comprising one or more cysteine residues for attachment of PEG may be fused to the N- and/or C-terminus of a Nanobody of the invention, all using techniques of protein engineering known per se to the skilled person.

Preferably, for the Nanobodies and proteins of the invention, a PEG is used with a molecular weight of more than 5000, such as more than 10,000 and less than 200,000, such as less than 100,000; for example in the range of 20,000-80,000.

With regard to pegylation, its should be noted that generally, the invention also encompasses any Nanobody of the invention and/or polypeptide of the invention that has been pegylated at one or more amino acid positions, preferably in such a way that said pegylation either (1) increases the half-life in vivo; (2) reduces immunogenicity; (3) provides one or more further beneficial properties known per se for pegylation; (4) does not essentially affect the affinity of the Nanobody and/or polypeptide for TNF-alpha (e.g. does not reduce said affinity by more than 90%, preferably not by more than 50 %, and more preferably not

153

by more than 10%, as determined by a suitable assay, such as those described in the Examples below); and/or (4) does not affect any of the other desired properties of the Nanobodies and/or polypeptides of the invention. Suitable PEG-groups and methods for attaching them, either specifically or non-specifically, will be clear to the skilled person. Suitable kits and reagents for such pegylation can for example be obtained from Nektar (CA, USA).

Another, usually less preferred modification comprises N-linked or O-linked glycosylation, usually as part of co-translational and/or post-translational modification, depending on the host cell used for expressing the Nanobody or polypeptide of the invention.

Yet another modification may comprise the introduction of one or more detectable labels or other signal-generating groups or moieties, depending on the intended use of the labelled Nanobody. Suitable labels and techniques for attaching, using and detecting them will be clear to the skilled person, and for example include, but are not limited to, fluorescent labels (such as fluorescein, isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde, and fluorescamine and fluorescent metals such as ^{152}Eu or others metals from the lanthanide series), phosphorescent labels, chemiluminescent labels or bioluminescent labels (such as luminal, isoluminol, theromatic acridinium ester, imidazole, acridinium salts, oxalate ester, dioxetane or GFP and its analogs), radio-isotopes (such as ^{3}H, ^{125}I, ^{32}P, ^{35}S, ^{14}C, ^{51}Cr, ^{36}Cl, ^{57}Co, ^{58}Co, ^{59}Fe, and ^{75}Se), metals, metals chelates or metallic cations (for example metallic cations such as ^{99m}Tc, ^{123}I, ^{111}In, ^{131}I, ^{97}Ru, ^{67}Cu, ^{67}Ga, and ^{68}Ga or other metals or metallic cations that are particularly suited for use in *in vivo*, *in vitro* or *in situ* diagnosis and imaging, such as (^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Cr, and ^{56}Fe), as well as chromophores and enzymes (such as malate dehydrogenase, staphylococcal nuclease, delta-V-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, biotinavidin peroxidase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, β-galactosidase, ribonuclease, urease, catalase, glucose-VI-phosphate dehydrogenase, glucoamylase and acetylcholine esterase). Other suitable labels will be clear to the skilled person, and for example include moieties that can be detected using NMR or ESR spectroscopy.

Such labelled Nanobodies and polypeptides of the invention may for example be used for in vitro, in vivo or in situ assays (including immunoassays known per se such as ELISA, RIA, EIA and other "sandwich assays", etc.) as well as in vivo diagnostic and imaging purposes, depending on the choice of the specific label.

As will be clear to the skilled person, another modification may involve the introduction of a chelating group, for example to chelate one of the metals or metallic cations referred to above. Suitable chelating groups for example include, without limitation, diethyl-enetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

Yet another modification may comprise the introduction of a functional group that is one part of a specific binding pair, such as the biotin-(strept)avidin binding pair. Such a functional group may be used to link the Nanobody of the invention to another protein, polypeptide or chemical compound that is bound to the other half of the binding pair, i.e. through formation of the binding pair. For example, a Nanobody of the invention may be conjugated to biotin, and linked to another protein, polypeptide, compound or carrier conjugated to avidin or streptavidin. For example, such a conjugated Nanobody may be used as a reporter, for example in a diagnostic system where a detectable signal-producing agent is conjugated to avidin or streptavidin. Such binding pairs may for example also be used to bind the Nanobody of the invention to a carrier, including carriers suitable for pharmaceutical purposes. One non-limiting example are the liposomal formulations described by Cao and Suresh, Journal of Drug Targetting, 8, 4, 257 (2000). Such binding pairs may also be used to link a therapeutically active agent to the Nanobody of the invention.

For some applications, in particular for those applications in which it is intended to kill a cell that expresses the target against which the Nanobodies of the invention are directed (e.g. in the treatment of cancer), or to reduce or slow the growth and/or proliferation such a cell, the Nanobodies of the invention may also be linked to a toxin or to a toxic residue or moiety. Examples of toxic moieties, compounds or residues which can be linked to a Nanobody of the invention to provide – for example – a cytotoxic compound will be clear to the skilled person and can for example be found in the prior art cited above and/or in the further description herein. One example is the so-called ADEPT™ technology WO 03/055527.

Other potential chemical and enzymatical modifications will be clear to the skilled person. Such modifications may also be introduced for research purposes (e.g. to study function-activity relationships). Reference is for example made to Lundblad and Bradshaw, Biotechnol. Appl. Biochem., 26, 143-151 (1997).

As mentioned above, the invention also relates to proteins or polypeptides comprising at least one V_{HH} domain (i.e. as identified using the methods of the invention) or at least one Nanobody based thereon.

According to one non-limiting embodiment of the invention, such a polypeptide of the invention essentially consists of a Nanobody. By "essentially consist of" is meant that the amino acid sequence of the polypeptide of the invention either is exactly the same as the amino acid sequence of a Nanobody (as mentioned above) or corresponds to the amino acid sequence of a Nanobody in which a limited number of amino acid residues, such as 1-10 amino acid residues and preferably 1-6 amino acid residues, such as 1, 2, 3, 4, 5 or 6 amino acid residues, have been added to the amino terminal end, to the carboxy terminal end, or both to the amino terminal end and to the carboxy terminal end of the amino acid sequence of the Nanobody.

Said amino acid residues may or may not change, alter or otherwise influence the (biological) properties of the Nanobody and may or may not add further functionality to the Nanobody. For example, such amino acid residues:

a) can comprise an N-terminal Met residue, for example as result of expression in a heterologous host cell or host organism.

b) may form a signal sequence or leader sequence that directs secretion of the Nanobody from a host cell upon synthesis. Suitable secretory leader peptides will be clear to the skilled person, and may be as further described herein. Usually, such a leader sequence will be linked to the N-terminus of the Nanobody, although the invention in its broadest sense is not limited thereto;

c) may form a sequence or signal that allows the Nanobody to be directed towards and/or to penetrate or enter into specific organs, tissues, cells, or parts or compartments of cells, and/or that allows the Nanobody to penetrate or cross a biological barrier such as a cell membrane, a cell layer such as a layer of epithelial cells, a tumor including solid tumors, or the blood-brain-barrier. Examples of such amino acid sequences will be clear to the skilled person. Some non-limiting examples are the small peptide vectors ("Pep-trans vectors") described in WO 03/026700 and in Temsamani et al., Expert Opin. Biol. Ther., 1, 773 (2001); Temsamani and Vidal, Drug Discov. Today, 9, 1012 (004) and Rousselle, J. Pharmacol. Exp. Ther., 296, 124-131 (2001), and the membrane translocator sequence described by Zhao et al., Apoptosis, 8, 631-637 (2003). C-terminal and N-terminal amino acid sequences for intracellular targeting of antibody fragments are for example described by Cardinale et al., Methods, 34, 171 (2004). Other suitable techniques for intracellular targeting involve the expression and/or use of so-called "intrabodies" comprising a Nanobody of the invention, as mentioned below;

d) may form a "tag", for example an amino acid sequence or residue that allows or facilitates the purification of the Nanobody, for example using affinity techniques directed against said sequence or residue. Thereafter, said sequence or residue may be removed (e.g. by chemical or enzymatical cleavage) to provide the Nanobody sequence (for this purpose, the tag may optionally be linked to the Nanobody sequence via a cleavable linker sequence or contain a cleavable motif). Some preferred, but non-limiting examples of such residues are multiple histidine residues, glutatione residues and a myc-tag such as AAAEQKLISEEDLNGAA [SEQ ID NO:31];

e) may be one or more amino acid residues that have been functionalized and/or that can serve as a site for attachment of functional groups. Suitable amino acid residues and functional groups will be clear to the skilled person and include, but are not limited to, the amino acid residues and functional groups mentioned herein for the derivatives of the Nanobodies of the invention.

According to another embodiment, a polypeptide of the invention comprises a Nanobody of the invention, which is fused at its amino terminal end, at its carboxy terminal end, or both at its amino terminal end and at its carboxy terminal end to at least one further amino acid sequence, i.e. so as to provide a fusion protein comprising said Nanobody of the invention and the one or more further amino acid sequences. Such a fusion will also be referred to herein as a "Nanobody fusion".

The one or more further amino acid sequence may be any suitable and/or desired amino acid sequences. The further amino acid sequences may or may not change, alter or otherwise influence the (biological) properties of the Nanobody, and may or may not add further functionality to the Nanobody or the polypeptide of the invention. Preferably, the further amino acid sequence is such that it confers one or more desired properties or functionalities to the Nanobody or the polypeptide of the invention.

Example of such amino acid sequences will be clear to the skilled person, and may generally comprise all amino acid sequences that are used in peptide fusions based on conventional antibodies and fragments thereof (including but not limited to ScFv's and single domain antibodies). Reference is for example made to the review by Holliger and Hudson, Nature Biotechnology, 23, 9, 1126-1136 (2005),

For example, such an amino acid sequence may be an amino acid sequence that increases the half-life, the solubility, or the absorption, reduces the immunogenicity or the toxicity, eliminates or attenuates undesirable side effects, and/or confers other advantageous

properties to and/or reduces the undesired properties of the polypeptides of the invention, compared to the Nanobody of the invention per se. Some non-limiting examples of such amino acid sequences are serum proteins, such as human serum albumin (see for example WO 00/27435) or haptenic molecules (for example haptens that are recognized by circulating antibodies, see for example WO 98/22141).

The further amino acid sequence may also provide a second binding site, which binding site may be directed against any desired protein, polypeptide, antigen, antigenic determinant or epitope (including but not limited to the same protein, polypeptide, antigen, antigenic determinant or epitope against which the Nanobody of the invention is directed, or a different protein, polypeptide, antigen, antigenic determinant or epitope). For example, the further amino acid sequence may provide a second binding site that is directed against a serum protein (such as, for example, human serum albumin or another serum protein such as IgG), so as to provide increased half-life in serum. Reference is for example made to EP 0 368 684, WO 91/01743, WO 01/45746 and WO 04/003019 (in which various serum proteins are mentioned), the International application by applicant entitled "Nanobodies™ against amyloid-beta and polypeptides comprising the same for the treatment of degenerative neural diseases such as Alzheimer's disease" (in which various other proteins are mentioned), as well as to Harmsen et al., Vaccine, 23 (41); 4926-42.

According to another embodiment, the one or more further amino acid sequences may comprise one or more parts, fragments or domains of conventional 4-chain antibodies (and in particular human antibodies) and/or of heavy chain antibodies. For example, although usually less preferred, a Nanobody of the invention may be linked to a conventional (preferably human) V_H or V_L domain domain or to a natural or synthetic analog of a V_H or V_L domain, again optionally via a linker sequence (including but not limited to other (single) domain antibodies, such as the dAb's described by Ward et al.).

The at least one Nanobody may also be linked to one or more (preferably human) CH_1, CH_2 and/or CH_3 domains, optionally via a linker sequence. For instance, a Nanobody linked to a suitable CH_1 domain could for example be used - together with suitable light chains - to generate antibody fragments/structures analogous to conventional Fab fragments or F(ab')2 fragments, but in which one or (in case of an F(ab')2 fragment) one or both of the conventional V_H domains have been replaced by a Nanobody of the invention. Also, two Nanobodies could be linked to a CH3 domain (optionally via a linker) to provide a construct with increased half-life in vivo.

According to one specific embodiment of a polypeptide of the invention, one or more Nanobodies of the invention may linked to one or more antibody parts, fragments or domains that confer one or more effector functions to the polypeptide of the invention and/or may confer the ability to bind to one or more Fc receptors. For example, for this purpose, and without being limited thereto, the one or more further amino acid sequences may comprise one or more CH_2 and/or CH_3 domains of an antibody, such as from a heavy chain antibody (as described herein) and more preferably from a conventional human 4-chain antibody; and/or may form (part of) and Fc region, for example from IgG, from IgE or from another human Ig. For example, WO 94/04678 describes heavy chain antibodies comprising a Camelid V_{HH} domain or a humanized derivative thereof (i.e. a Nanobody), in which the Camelidae CH_2 and/or CH_3 domain have been replaced by human CH_2 and CH_3 domains, so as to provide an immunoglobulin that consists of 2 heavy chains each comprising a Nanobody and human CH2 and CH3 domains (but no CH1 domain), which immunoglobulin has the effector function provided by the CH2 and CH3 domains and which immunoglobulin can function without the presence of any light chains. Other amino acid sequences that can be suitably linked to the Nanobodies of the invention so as to provide an effector function will be clear to the skilled person, and may be chosen on the basis of the desired effector function(s). Reference is for example made to WO 04/058820, WO 99/42077 and WO 05/017148, as well as the review by Holliger and Hudson, supra. Coupling of a Nanobody of the invention to an Fc portion may also lead to an increased half-life, compared to the corresponding Nanobody of the invention. For some applications, the use of an Fc portion and/or of constant domains (i.e. CH_2 and/or CH_3 domains) that confer increased half-life without any biologically significant effector function may also be suitable or even preferred. Other suitable constructs comprising one or more Nanobodies and one or more constant domains with increased half-life in vivo will be clear to the skilled person, and may for example comprise two Nanobodies linked to a CH3 domain, optionally via a linker sequence. Generally, any fusion protein or derivatives with increased half-life will preferably have a molecular weight of more than 50 kD, the cut-off value for renal absorption.

The further amino acid sequences may also form a signal sequence or leader sequence that directs secretion of the Nanobody or the polypeptide of the invention from a host cell upon synthesis (for example to provide a pre-, pro- or prepro- form of the polypeptide of the invention, depending on the host cell used to express the polypeptide of the invention).

The further amino acid sequence may also form a sequence or signal that allows the Nanobody or polypeptide of the invention to be directed towards and/or to penetrate or enter into specific organs, tissues, cells, or parts or compartments of cells, and/or that allows the Nanobody or polypeptide of the invention to penetrate or cross a biological barrier such as a cell membrane, a cell layer such as a layer of epithelial cells, a tumor including solid tumors, or the blood-brain-barrier. Suitable examples of such amino acid sequences will be clear to the skilled person, and for example include, but are not limited to, the "Peptrans" vectors mentioned above, the sequences described by Cardinale et al. and the amino acid sequences and antibody fragments known per se that can be used to express or produce the Nanobodies and polypeptides of the invention as so-called "intrabodies", for example as described in WO 94/02610, WO 95/22618, US-A-7004940, WO 03/014960, WO 99/07414; WO 05/01690; EP 1 512 696; and in Cattaneo, A. & Biocca, S. (1997) Intracellular Antibodies: Development and Applications. Landes and Springer-Verlag; and in Kontermann, Methods 34, (2004), 163-170, and the further references described therein.

For some applications, in particular for those applications in which it is intended to kill a cell that expresses the target against which the Nanobodies of the invention are directed (e.g. in the treatment of cancer), or to reduce or slow the growth and/or proliferation such a cell, the Nanobodies of the invention may also be linked to a (cyto)toxic protein or polypeptide. Examples of such toxic proteins and polypeptides which can be linked to a Nanobody of the invention to provide – for example – a cytotoxic polypeptide of the invention will be clear to the skilled person and can for example be found in the prior art cited above and/or in the further description herein. One example is the so-called ADEPT™ technology WO 03/055527.

According to one non-limiting embodiment, one or more amino acid residues can be added to, inserted in and/or substituted in the amino acid sequence of a Nanobody or polypeptide of the invention, so as to provide one or more specific amino acid residues for attachment of a PEG-group.

The efficacy of protein pharmaceuticals depends on its potency to neutralize the target but also on the intrinsic pharmacokinetics of the potential drug. Because the kidney generally filters out molecules below 60,000 Daltons (Da), efforts to reduce clearance have focussed on increasing the molecular weight of the biopharmaceutical through protein fusions (Syed et al., 1997), glycosylations or modification with polyethylene glycol polymers, i.e., PEGylation (Lee et al., 1999; Abuchowski et al., 1977; Nucci et al., 1991; Lecolley, et al. Chem

Commun, 2004; Tao et al., J Am Chem Soc, 2004; Mantovani et al., 2005). These methods successfully extend the in vivo exposure of the biopharmaceutical.

Alternatively, the half-life can be extended using another pegylating agent, POLY PEG for conjugation to the bivalent Nanobodies,TNF56 or TNF55. POLY PEG are comb shape polymers with PEG teeth on a methacrylic backbone. POLY PEGs can vary on the length of the PEG chain, on the methacrylic backbone and on the active end-group which determines the method of conjugation of the POLY PEG to the Nanobody. Site-specific conjugation to the C-terminal cysteine present in the Nanobodies can be achieved through the active maleimide end-group in the POLY PEG.

The invention also encompasses any Nanobody of the invention and/or polypeptide of the invention that has been glycosylated at one or more amino acid positions, usually depending upon the host used to express the Nanobody or polypeptide of the invention (as further described below).

According to one non-limiting embodiment, one or more amino acid residues can be added to, inserted in and/or substituted in the amino acid sequence of a Nanobody or polypeptide of the invention, so as to provide one or more specific amino acid residues and/or a site that can be glycosylated by the host organism used. By means of a preferred, but non-limiting example, the N-residue on position 50 within CDR2 of a Nanobody of the invention can for example be replaced by a Q, D or S residue so as to provide a glycosylation site, e.g. for glycosylation by *Pichia*.

According to another embodiment, a polypeptide of the invention can comprise the amino acid sequence of a Nanobody, which is fused at its amino terminal end, at its carboxy terminal end, or both at its amino terminal end and at its carboxy terminal end with at least one further amino acid sequence.

Again, said further amino acid sequence(s) may or may not change, alter or otherwise influence the (biological) properties of the Nanobody and may or may not add further functionality to the Nanobody.

For example, according to one preferred, but non-limiting embodiment, said further amino acid sequence may comprise at least one further Nanobody, so as to provide a polypeptide of the invention that comprises at least two, such as three, four or five, Nanobodies, in which said Nanobodies may optionally be linked via one or more linker sequences (as defined herein).

Polypeptides of the invention comprising two or more Nanobodies will also referred to herein as "multivalent" polypeptides. For example a "bivalent" polypeptide of the Invention comprises two Nanobodies, optionally linked via a linker sequence, whereas a "trivalent" polypeptide of the invention comprises three Nanobodies, optionally linked via two linker sequences; etc.

In a multivalent polypeptide of the invention, the two or more Nanobodies may be the same or different. For example, the two or more Nanobodies in a multivalent polypeptide of the invention:

- may be directed against the same antigen, i.e. against the same parts or epitopes of said antigen or against two or more different parts or epitopes of said antigen; and/or:
- may be directed against the different antigens;

or a combination thereof.

Thus, a bivalent polypeptide of the invention for example:

- may comprise two identical Nanobodies;
- may comprise a first Nanobody directed against a first part or epitope of an antigen and a second Nanobody directed against the same part or epitope of said antigen or against another part or epitope of said antigen;
- or may comprise a first Nanobody directed against a first antigen and a second Nanobody directed against a second antigen different from said first antigen;

whereas a trivalent Polypeptide of the Invention for example:

- may comprises three identical or different Nanobodies directed against the same or different parts or epitopes of the same antigen;
- may comprise two identical or different Nanobodies directed against the same or different parts or epitopes on a first antigen and a third Nanobody directed against a second antigen different from said first antigen; or
- may comprise a first Nanobody directed against a first antigen, a second Nanobody directed against a second antigen different from said first antigen, and a third Nanobody directed against a third antigen different from said first and second antigen,

Polypeptides of the invention that contain at least two Nanobodies, in which at least one Nanobody is directed against a first antigen and at least one Nanobody is directed against a second Nanobody different from the first antigen, will also be referred to as "multispecific" Nanobodies. Thus, a "bispecific" Nanobody is a Nanobody that comprises at least one Nanobody directed against a first antigen and at least one further Nanobody directed against a

second antigen, whereas a "trispecific" Nanobody is a Nanobody that comprises at least one Nanobody directed against a first antigen, at least one further Nanobody directed against a second antigen, and at least one further Nanobody directed against a third antigen; etc.

Accordingly, in their simplest form, a bispecific polypeptide of the invention is a bivalent polypeptide of the invention (as defined herein), comprising a first Nanobody directed against a first antigen and a second Nanobody directed against a second antigen, in which said first and second Nanobody may optionally be linked via a linker sequence (as defined herein); whereas a trispecific polypeptide of the invention in its simplest form is a trivalent polypeptide of the invention (as defined herein), comprising a first Nanobody directed against a first antigen, a second Nanobody directed against a second antigen and a third Nanobody directed against a third antigen, in which said first, second and third Nanobody may optionally be linked via one or more, and in particular one and more in particular two, linker sequences.

However, as will be clear from the description hereinabove, the invention is not limited thereto, in the sense that a multispecific polypeptide of the invention may comprise any number of Nanobodies directed against two or more different antigens.

For multivalent and multispecific polypeptides containing one or more V_{HH} domains and their preparation, reference is also made to Conrath et al., *J. Biol. Chem.*, Vol. 276, 10. 7346-7350, as well as to EP 0 822 985.

In the polypeptides of the invention, the one or more Nanobodies and the one or more polypeptides may be directly linked to each other (as for example described in WO 99/23221) and/or may be linked to each other via one or more suitable spacers or linkers, or any combination thereof.

Suitable spacers or linkers for use in multivalent and multispecific polypeptides will be clear to the skilled person, and may generally be any linker or spacer used in the art to link amino acid sequences. Preferably, said linker or spacer is suitable for use in constructing proteins or polypeptides that are intended for pharmaceutical use.

Some particularly preferred spacers include the spacers and linkers that are used in the art to link antibody fragments or antibody domains. These include the linkers mentioned in the general background art cited above, as well as for example linkers that are used in the art to construct diabodies or ScFv fragments (in this respect, however, its should be noted that, whereas in diabodies and in ScFv fragments, the linker sequence used should have a length, a degree of flexibility and other properties that allow the pertinent V_H and V_L domains to come

together to form the complete antigen-binding site, there is no particular limitation on the length or the flexibility of the linker used in the polypeptide of the invention, since each Nanobody by itself forms a complete antigen-binding site).

Other suitable linkers generally comprise organic compounds or polymers, in particular those suitable for use in proteins for pharmaceutical use. For instance, poly(ethyleneglycol) moieties have been used to link antibody domains, see for example WO 04/081026.

It is also within the scope of the invention that the linker(s) used confer one or more other favourable properties or functionality to the polypeptides of the invention, and/or provide one or more sites for the formation of derivatives and/or for the attachment of functional groups (e.g. as described herein for the derivatives of the Nanobodies of the invention). For example, linkers containing one or more charged amino acid residues (see Table A-3 above) can provide improved hydrophilic properties, whereas linkers that form or contain small epitopes or tags can be used for the purposes of detection, identification and/or purification. Again, based on the disclosure herein, the skilled person will be able to determine the optimal linkers for use in a specific polypeptide of the invention, optionally after on some limited routine experiments.

Finally, when two or more linkers are used in the polypeptides of the invention, these linkers may be the same or different. Again, based on the disclosure herein, the skilled person will be able to determine the optimal linkers for use in a specific polypeptide of the invention, optionally after on some limited routine experiments.

Linkers for use in multivalent and multispecific polypeptides will be clear to the skilled person, and for example include gly-ser linkers, for example of the type $(gly_xser_y)_z$, such as (for example $(gly_4ser)_3$ or $(gly_3ser_2)_3$, as described in WO 99/42077, hinge like regions such as the hinge regions of naturally occurring heavy chain antibodies or similar sequences. For other suitable linkers, reference is also made to the general background art cited above. Some particularly preferred linkers are given in SEQ ID NO's 68 and 69.

Linkers can also provide some functionality for the multivalent or multispecific polypeptide. For example, linkers containing one or more charged amino acid residues (see Table 1 above) can provide improved hydrophilic properties, whereas linkers that form or contain small epitopes or tags can be used for the purposes of detection, identification and/or purification.

As mentioned herein, in a protein or polypeptide of the invention, the anti-TNF Nanobodies mentioned herein are preferably linked in such a way that said protein or polypeptide, upon binding to a TNF trimer, is capable inhibiting or reducing the TNF receptor crosslinking that is mediated by said TNF trimer and/or the signal transduction that is mediated by such receptor crosslinking; and/or in such a way that the protein or polypeptide is capable of intramolecular binding to at least two TNF receptor binding sites on a TNF trimer. Suitable linkers are as described herein.

As also mentioned herein, whether a protein or polypeptide provides intermolecular binding or extramolecular binding can be assessed (at least initially) by a size exclusion chromatography. By Size Exclusion Chromatography the complexes of TNF-alpha and antibodies can be analyzed for determining the number and the ratio of antibody and TNF-alpha molecules in the complex. From these data it can be deduced if inter- or intramolecular binding occurs, as was done by Santora and colleagues (Santora, L.C., et al, Anal Biochem. 2001) for establishing the stoichiometry of binding of monoclonal antibody D2E7 (Humira) to TNF-alpha at different ratios of antibody and target. From the molecular weight of the complex it was concluded that three antibody molecules complexed with three TNF trimers, thereby indicating that the antibody binds in an intermolecular mode. Similar experiments were performed with bivalent Nanobodies, in which a very short linker induced the formation of large molecular complexes, which were obtained by intermolecular bonds. However, the same bivalent Nanobodies constructs with longer linkers eluted from the gel filtration column as discrete small complexes, thereby demonstrating that intramolecular bonds were formed. Combined with the bioassay data, in which the longer linker containing Nanobody TNF1 had an optimal potency (complete neutralization of amount of TNF used in the assay, i.e. 10 pM), it can be concluded that intramolecular binding of the bivalent Nanobody efficiently prevents cross-linking of two cell bound receptors and the associated receptor activation. Known monoclonal antibodies such as Humira or Remicade can not form such intramolecular bonds, leaving always two receptor bindingsites on the trimeric TNF molecule to a certain degree available for interaction with cell bound receptor, which translates into less potent neutralization as measured in the bioassay.

Alternatively, whether a protein or polypeptide provides intermolecular binding or extramolecular binding can be assessed by crystallography and/or molecular modelling (or other suitable in silico techniques). A model of a trimeric TNF30/TNF-alpha complex was generated based on the crystal structure of the monomeric wild type TNF1/TNF-alpha

complex. From this structure the final TNF30-linker-ALB8-linker-TNF30 construct was modeled. The TNF30-linker-ALB8-linker-TNF30 construct was modelled starting from the trimer of TNFα with two TNF30 molecules bound. As the structure of the ALB8 is not known, a third TNF30 molecule was used instead, which was placed in between the other two Nanobodies along the line between the N- and C-termini. The 9 amino acid linkers were then added manually.

The model is shown in Figure 62. Clearly, the 9 amino acid linkers together with the ALB8 provide ample room to span the about 66 Å between the two TNF30 domains bound to TNFα. ALB8 by itself already spans 40 Å, and each linker can span another ~27 Å in completely extended conformation. As a result, the ALB8 has quite some flexibility of movement, and it is not expected that its binding to albumin would interfere much with the binding to TNFα.

Moreover, it is likely that the linkers can be shortened without affecting avidity, especially in the case of the linker that is C-terminal of ALB8. This may have the benificial effect of increased binding to the same TNFα trimer versus crosslinking trimers, because the probability that the second TNF30 associates with a different TNFα increases with the length of the linker.

As also further described herein, a multispecific polypeptide of the invention directed against a desired antigen and against at least one serum protein, such as the serum proteins mentioned hereinbelow, and in particular against human serum albumin, may show increased half-life in serum, compared to the corresponding monovalent Nanobody.

As mentioned hereinabove, the methods described herein are particularly suited for generating such multivalent of multispecific polypeptides of the invention.

In a polypeptide of the invention, the at least one Nanobody may also be linked to a conventional V_H domain or to a natural or synthetic analog of a V_H domain, optionally via a linker sequence.

In a polypeptide of the invention, the at least one Nanobody may also be linked to a V_L domain or to a natural or synthetic analog of a V_L domain, optionally via a linker sequence, so as to provide a polypeptide of the invention that is in the form analogous to a conventional scFv fragment, but containing a Nanobody instead of a V_H domain.

In a polypeptide of the invention, the at least one Nanobody may also be linked to one or more of a CH1, CH2 and/or CH3 domain, optionally via a linker sequence. For instance, a Nanobody linked to a suitable CH1 domain could for example be used - together with

suitable light chains - to generate antibody fragments/structures analogous to conventional Fab fragments or $F(ab')_2$ fragments, but in which one or (in case of an $F(ab')_2$ fragment) one or both of the conventional V_H domains have been replaced by a Nanobody. Such fragments may also be heterospecific or bispecific, i.e. directed against two or more antigens. A Nanobody linked to suitable CH2 and CH3 domains, for example derived from Camelids, could be used to form a monospecific or bispecific heavy chain antibody. Finally, a Nanobody linked to suitable CH1, CH2 and CH3 domains, for example derived from a human being, could be used – together with suitable light chains - to form an antibody that is analogous to a conventional 4-chain antibody, but in which one or both of the conventional V_H domains have been replaced by a Nanobody.

Also, in addition to the one or more Nanobodies, Polypeptides of the Invention can also contain functional groups, moieties or residues, for example therapeutically active substances, such as those mentioned below, and/or markers or labels, such as fluorescent markers, isotopes, etc., as further described hereinbelow.

The Nanobodies of the invention, the polypeptides of the invention, and nucleic acids encoding the same, can be prepared in a manner known per se, as will be clear to the skilled person from the further description herein. Some preferred, but non-limiting methods for preparing the Nanobodies, polypeptides and nucleic acids include the methods and techniques mentioned above and/or further described hereinbelow .

As will be clear to the skilled person, one particularly useful method for preparing a Nanobody and/or a polypeptide of the invention generally comprises the steps of:

- the expression, in a suitable host cell or host organism (also referred to herein as a "*host of the invention*") or in another suitable expression system of a nucleic acid that encodes said Nanobody or polypeptide of the invention (also referred to herein as a "*nucleic acid of the invention*"), optionally followed by:
- isolating and/or purifying the Nanobody or polypeptide of the invention thus obtained.

In particular, such a method may comprise the steps of:

- cultivating and/or maintaining a host of the invention under conditions that are such that said host of the invention expresses and/or produces at least one Nanobody and/or polypeptide of the invention; optionally followed by:
- isolating and/or purifying the Nanobody or polypeptide of the invention thus obtained.

A nucleic acid of the invention can be in the form of single or double stranded DNA or RNA, and is preferably in the form of double stranded DNA. For example, the nucleotide

sequences of the invention may be genomic DNA, cDNA or synthetic DNA (such as DNA with a codon usage that has been specifically adapted for expression in the intended host cell or host organism).

According to one embodiment of the invention, the nucleic acid of the invention is in essentially isolated from, as defined hereinabove.

The nucleic acid of the invention may also be in the form of, be present in and/or be part of a vector, such as for example a plasmid, cosmid or YAC, which again may be in essentially isolated form.

The nucleic acids of the invention can be prepared or obtained in a manner known per se, based on the information on the amino acid sequences for the polypeptides of the invention given herein, and/or can be isolated from a suitable natural source. To provide analogs, nucleotide sequences encoding naturally occurring V_{HH} domains can for example be subjected to site-directed mutagenesis, so at to provide a nucleic acid of the invention encoding said analog. Also, as will be clear to the skilled person, to prepare a nucleic acid of the invention, also several nucleotide sequences, such as at least one nucleotide sequence encoding a Nanobody and for example nucleic acids encoding one or more linkers can be linked together in a suitable manner.

Techniques for generating the nucleic acids of the invention will be clear to the skilled person and may for instance include, but are not limited to, automated DNA synthesis; site-directed mutagenesis; combining two or more naturally occurring and/or synthetic sequences (or two or more parts thereof), introduction of mutations that lead to the expression of a truncated expression product; introduction of one or more restriction sites (e.g. to create casettes and/or regions that may easily be digested and/or ligated using suitable restriction enzymes), and/or the introduction of mutations by means of a PCR reaction using one or more "mismatched" primers, using for example a sequence of a naturally occurring GPCR as a template. These and other techniques will be clear to the skilled person, and reference is again made to the standard handbooks, such as Sambrook et al. and Ausubel et al., mentioned above, as well as the Examples below.

The nucleic acid of the invention may also be in the form of, be present in and/or be part of a genetic construct, as will be clear to the person skilled in the art. Such genetic constructs generally comprise at least one nucleic acid of the invention that is optionally linked to one or more elements of genetic constructs known per se, such as for example one or more suitable regulatory elements (such as a suitable promoter(s), enhancer(s),

terminator(s), etc.) and the further elements of genetic constructs referred to hereinbelow. Such genetic constructs comprising at least one nucleic acid of the invention will also be referred to herein as "*genetic constructs of the invention*".

The genetic constructs of the invention may be DNA or RNA, and are preferably double-stranded DNA. The genetic constructs of the invention may also be in a form suitable for transformation of the intended host cell or host organism, in a form suitable for integration into the genomic DNA of the intended host cell or in a form suitable independent replication, maintenance and/or inheritance in the intended host organism. For instance, the genetic constructs of the invention may be in the form of a vector, such as for example a plasmid, cosmid, YAC, a viral vector or transposon. In particular, the vector may be an expression vector, i.e. a vector that can provide for expression *in vitro* and/or *in vivo* (e.g. in a suitable host cell, host organism and/or expression system).

In a preferred but non-limiting embodiment, a genetic construct of the invention comprises

a) at least one nucleic acid of the invention; operably connected to

b) one or more regulatory elements, such as a promoter and optionally a suitable terminator;

c) and optionally also

d) one or more further elements of genetic constructs known per se;

in which the terms "regulatory element", "promoter", "terminator" and "operably connected" have their usual meaning in the art (as further described below); and in which said "further elements" present in the genetic constructs may for example be 3'- or 5'-UTR sequences, leader sequences, selection markers, expression markers/reporter genes, and/or elements that may facilitate or increase (the efficiency of) transformation or integration. These and other suitable elements for such genetic constructs will be clear to the skilled person, and may for instance depend upon the type of construct used, the intended host cell or host organism; the manner in which the nucleotide sequences of the invention of interest are to be expressed (e.g. via constitutive, transient or inducible expression); and/or the transformation technique to be used.

Preferably, in the genetic constructs of the invention, said at least one nucleic acid of the invention and said regulatory elements, and optionally said one or more further elements, are "operably linked" to each other, by which is generally meant that they are in a functional relationship with each other. For instance, a promoter is considered "operably linked" to a

coding sequence if said promoter is able to initiate or otherwise control/regulate the transcription and/or the expression of a coding sequence (in which said coding sequence should be understood as being "under the control of" said promotor). Generally, when two nucleotide sequences are operably linked, they will be in the same orientation and usually also in the same reading frame. They will usually also be essentially contiguous, although this may also not be required.

Preferably, the regulatory and further elements of the genetic constructs of the invention are such that they are capable of providing their intended biological function in the intended host cell or host organism.

For instance, a promoter, enhancer or terminator should be "*operable*" in the intended host cell or host organism, by which is meant that (for example) said promoter should be capable of initiating or otherwise controlling/regulating the transcription and/or the expression of a nucleotide sequence - e.g. a coding sequence - to which it is operably linked (as defined herein).

Some particularly preferred promoters include, but are not limited to, promoters known per se for the expression in bacterial cells, such as those mentioned hereinbelow and/or those used in the Examples.

A selection marker should be such that it allows - i.e. under appropriate selection conditions - host cells and/or host organisms that have been (succesfully) transformed with the nucleotide sequence of the invention to be distinguished from host cells/organisms that have not been (succesfully) transformed. Some preferred, but non-limiting examples of such markers are genes that provide resistance against antibiotics (such as kanamycine or ampicilline), genes that provide for temperature resistance, or genes that allow the host cell or host organism to be maintained in the absence of certain factors, compounds and/or (food) components in the medium that are essential for survival of the non-transformed cells or organisms.

A leader sequence should be such that - in the intended host cell or host organism - it allows for the desired post-translational modifications and/or such that it directs the transcribed mRNA to a desired part or organelle of a cell. A leader sequence may also allow for secretion of the expression product from said cell. As such, the leader sequence may be any pro-, pre-, or prepro-sequence operable in the host cell or host organism. Leader sequences may not be required for expression in a bacterial cell.

An expression marker or reporter gene should be such that - in the host cell or host organism - it allows for detection of the expression of (a gene or nucleotide sequence present on) the genetic construct. An expression marker may optionally also allow for the localisation of the expressed product, e.g. in a specific part or organelle of a cell and/or in (a) specific cell(s), tissue(s), organ(s) or part(s) of a multicellular organism. Such reporter genes may also be expressed as a protein fusion with the amino acid sequence of the invention. Some preferred, but non-limiting examples include fluorescent proteins such as GFP.

Some preferred, but non-limiting examples of suitable promoters, terminator and further elements include those used in the Examples below. For some (further) non-limiting examples of the promoters, selection markers, leader sequences, expression markers and further elements that may be present/used in the genetic constructs of the invention - such as terminators, transcriptional and/or translational enhancers and/or integration factors - reference is made to the general handbooks such as Sambrook et al. and Ausubel et al. mentioned above, as well as to the examples that are given in WO 95/07463, WO 96/23810, WO 95/07463, WO 95/21191, WO 97/11094, WO 97/42320, WO 98/06737, WO 98/21355, US-A-6,207,410, US-A- 5,693,492 and EP 1 085 089. Other examples will be clear to the skilled person. Reference is also made to the general background art cited above and the further references cited hereinbelow.

The genetic constructs of the invention may generally be provided by suitably linking the nucleotide sequence(s) of the invention to the one or more further elements described above, for example using the techniques described in the general handbooks such as Sambrook et al. and Ausubel et al., mentioned above.

Often, the genetic constructs of the invention will be obtained by inserting a nucleotide sequence of the invention in a suitable (expression) vector known per se. Some preferred, but non-limiting examples of suitable expression vectors are those used in the Examples below, as well as those mentioned below.

The nucleic acids of the invention and/or the genetic constructs of the invention may be used to transform a host cell or host organism. The host or host cell cell may be any suitable (fungal, prokaryotic or eukaryotic) cell or cell line or any suitable fungal, prokaryotic or eukaryotic organism, for example:

- a bacterial strain, including but not limited to gram-negative strains such as strains of *Escherichia coli*; of *Proteus*, for example of *Proteus mirabilis*; of *Pseudomonas*, for example of *Pseudomonas fluorescens*; and gram-positive strains such as strains of

Bacillus, for example of *Bacillus subtilis* or of *Bacillus brevis*; of *Streptomyces*, for example of *Streptomyces lividans*; of *Staphylococcus*, for example of *Staphylococcus carnosus*; and of *Lactococcus*, for example of *Lactococcus lactis*;

- a fungal cell, including but not limited to cells from species of *Trichoderma*, for example from *Trichoderma reesei*; of *Neurospora*, for example from *Neurospora crassa*; of *Sordaria*, for example from *Sordaria macrospora*; of *Aspergillus*, for example from *Aspergillus niger* or from *Aspergillus sojae*; or from other filamentous fungi;
- a yeast cell, including but not limited to cells from species of *Saccharomyces*, for example of *Saccharomyces cerevisiae*; of *Schizosaccharomyces*, for example of *Schizosaccharomyces pombe*; of *Pichia*, for example of *Pichia pastoris* or of *Pichia methanolica*; of *Hansenula*, for example of *Hansenula polymorpha*; of *Kluyveromyces*, for example of *Kluyveromyces lactis*; of *Arxula*, for example of *Arxula adeninivorans*; of *Yarrowia*, for example of *Yarrowia lipolytica*;
- an amphibian cell or cell line, such as *Xenopus oocytes*;
- an insect-derived cell or cell line, such as cells/cell lines derived from lepidoptera, including but not limited to *Spodoptera* SF9 and Sf21 cells or cells/cell lines derived from *Drosophila*, such as Schneider and Kc cells;
- a plant or plant cell, for example in tobacco plants; and/or
- a mammalian cell or cell line, for example derived a cell or cell line derived from a human, from the mammals including but not limited to CHO-cells, BHK-cells (for example BHK-21 cells) and human cells or cell lines such as HeLa, COS (for example COS-7) and PER.C6 cells;

as well as all other hosts or host cells known per se for the expression and production of antibodies and antibody fragments (including but not limited to (single) domain antibodies and ScFv fragments), which will be clear to the skilled person. Reference is also made to the general background art cited hereinabove, as well as to for example WO 94/29457; WO 96/34103; WO 99/42077; Frenken et al., (1998), supra; Riechmann and Muyldermans, (1999), supra; van der Linden, (2000), supra; Thomassen et al., (2002), supra; Joosten et al., (2003), supra; Joosten et al., (2005), supra; and the further references cited herein.

The Nanobodies and polypeptides of the invention can also be introduced and expressed in one or more cells, tissues or organs of a multicellular organism, for example for prophylactic and/or therapeutic purposes (e.g. as a gene therapy). For this purpose, the

nucleotide sequences of the invention may be introduced into the cells or tissues in any suitable way, for example as such (e.g. using liposomes) or after they have been inserted into a suitable gene therapy vector (for example derived from retroviruses such as adenovirus, or parvoviruses such as adeno-associated virus). As will also be clear to the skilled person, such gene therapy may be performed *in vivo* and/or *in situ* in the body of a patent by administering a nucleic acid of the invention or a suitable gene therapy vector encoding the same to the patient or to specific cells or a specific tissue or organ of the patient; or suitable cells (often taken from the body of the patient to be treated, such as explanted lymphocytes, bone marrow aspirates or tissue biopsies) may be treated in vitro with a nucleotide sequence of the invention and then be suitably (re-)introduced into the body of the patient. All this can be performed using gene therapy vectors, techniques and delivery systems which are well known to the skilled person, for Culver, K. W., "Gene Therapy", 1994, p. xii, Mary Ann Liebert, Inc., Publishers, New York, N.Y). Giordano, Nature F Medicine 2 (1996), 534-539; Schaper, Circ. Res. 79 (1996), 911-919; Anderson, Science 256 (1992),808-813; Verma, Nature 389 (1994),239; Isner, Lancet 348 (1996),370-374; Muhlhauser, Circ. Res. 77 (1995),1077-1086; Onodera, Blood 91; (1998),30- 36; Verma, Gene Ther. 5 (1998),692-699; Nabel, Ann. N.Y. Acad. Sci. : 811 (1997), 289-292; Verzeletti, Hum. Gene Ther. 9 (1998), 2243-51; Wang, Nature Medicine 2 (1996),714-716; WO 94/29469; WO 97/00957, US 5,580,859; 1 US 5,5895466; or Schaper, Current Opinion in Biotechnology 7 (1996), 635-640. For example, in situ expression of ScFv fragments (Afanasieva et al., Gene Ther., 10, 1850-1859 (2003)) and of diabodies (Blanco et al., J. Immunol, 171, 1070-1077 (2003)) has been described in the art.

For expression of the Nanobodies in a cell, they may also be expressed as so-called or as so-called "intrabodies", as for example described in WO 94/02610, WO 95/22618 and US-A-7004940; WO 03/014960; in Cattaneo, A. & Biocca, S. (1997) Intracellular Antibodies: Development and Applications. Landes and Springer-Verlag; and in Kontermann, Methods 34, (2004), 163-170.

For production, the Nanobodies and polypeptides of the invention can for example also be produced in the milk of transgenic mammals, for example in the milk of rabbits, cows, goats or sheep (see for example US-A-6,741,957, US-A-6,304,489 and US-A-6,849,992 for general techniques for introducing transgenes into mammals), in plants or parts of plants including but not limited to their leaves, flowers, fruits, seed, roots or turbers (for

example in tobacco, maize, soybean or alfalfa) or in for example pupae of the silkworm Bombix mori.

Furthermore, the Nanobodies and polypeptides of the invention can also be expressed and/or produced in cell-free expression systems, and suitable examples of such systems will be clear to the skilled person. Some preferred, but non-limiting examples include expression in the wheat germ system; in rabbit reticulocyte lysates; or in the *E. coli* Zubay system.

As mentioned above, one of the advantages of the use of Nanobodies is that the polypeptides based thereon can be prepared through expression in a suitable bacterial system, and suitable bacterial expression systems, vectors, host cells, regulatory elements, etc., will be clear to the skilled person, for example from the references cited above. It should however be noted that the invention in its broadest sense is not limited to expression in bacterial systems.

Preferably, in the invention, an (in vivo or in vitro) expression system, such as a bacterial expression system, is used that provides the polypeptides of the invention in a form that is suitable for pharmaceutical use, and such expression systems will again be clear to the skilled person. As also will be clear to the skilled person, Polypeptides of the invention suitable for pharmaceutical use can be prepared using techniques for peptide synthesis.

For production on industrial scale, preferred heterologous hosts for the (industrial) production of Nanobodies or Nanobody-containing protein therapeutics include strains of *E. coli*, *Pichia pastoris*, *S. cerevisiae* that are suitable for large scale expression/production/fermentation, and in particular for large scale pharmaceutical expression/production/fermentation. Suitable examples of such strains will be clear to the skilled person. Such strains and production/expression systems are also made available by companies such as Biovitrum (Uppsala, Sweden).

Alternatively, mammalian cell lines, in particular Chinese hamster ovary (CHO) cells, can be used for large scale expression/production/fermentation, and in particular for large scale pharmaceutical expression/production/fermentation. Again, such expression/production systems are also made available by some of the companies mentioned above.

The choice of the specific expression system would depend in part on the requirement for certain post-translational modifications, more specifically glycosylation. The production of a Nanobody-containing recombinant protein for which glycosylation is desired or required would necessitate the use of mammalian expression hosts that have the ability to glycosylate the expressed protein. In this respect, it will be clear to the skilled person that the

glycosylation pattern obtained (i.e. the kind, number and position of residues attached) will depend on the cell or cell line that is used for the expression. Preferably, either a human cell or cell line is used (i.e. leading to a protein that essentially has a human glycosylation pattern) or another mammalian cell line is used that can provide a glycosylation pattern that is essentially and/or functionally the same as human glycosylation or at least mimics human glycosylation. Generally, prokaryotic hosts such as E. coli do not have the ability to glycosylate proteins, and the use of lower eukaryotes such as yeast are usually leads to a glycosylation pattern that differs from human glycosylation. Nevertheless, it should be understood that all the foregoing host cells and expression systems can be used in the invention, depending on the desired Nanobody or protein to be obtained.

Thus, according to one non-limiting embodiment of the invention, the Nanobody or polypeptide of the invention is glycosylated. According to another non-limiting embodiment of the invention, the Nanobody or polypeptide of the invention is non-glycosylated.

According to one preferred, but non-limiting embodiment of the invention, the Nanobody or polypeptide of the invention is produced in a bacterial cell, in particular a bacterial cell suitable for large scale pharmaceutical production, such as cells of the strains mentioned above.

According to another preferred, but non-limiting embodiment of the invention, the Nanobody or polypeptide of the invention is produced in a yeast cell, in particular a yeast cell suitable for large scale pharmaceutical production, such as cells of the species mentioned above.

According to yet another preferred, but non-limiting embodiment of the invention, the Nanobody or polypeptide of the invention is produced in a mammalian cell, in particular in a human cell or in a cell of a human cell line, and more in particular in a human cell or in a cell of a human cell line that is suitable for large scale pharmaceutical production, such as the cell lines mentioned hereinabove.

When expression in a host cell is used to produce the Nanobodies and the proteins of the invention, the Nanobodies and proteins of the invention can be produced either intracellullarly (e.g. in the cytosol, in the periplasma or in inclusion bodies) and then isolated from the host cells and optionally further purified; or can be produced extracellularly (e.g. in the medium in which the host cells are cultured) and then isolated from the culture medium and optionally further purified. When eukaryotic hosts cells are used, extracellular production is usually preferred since this considerably facilitates the further isolation and downstream

processing of the Nanobodies and proteins obtained. Bacterial cells such as the strains of E. coli mentioned above normally do not secrete proteins extracellularly, except for a few classes of proteins such as toxins and hemolysin, and secretory production in E. coli refers to the translocation of proteins across the inner membrane to the periplasmic space. Periplasmic production provides several advantages over cytosolic production. For example, the N-terminal amino acid sequence of the secreted product can be identical to the natural gene product after cleavage of the secretion signal sequence by a specific signal peptidase. Also, there appears to be much less protease activity in the periplasm than in the cytoplasm. In addition, protein purification is simpler due to fewer contaminating proteins in the periplasm. Another advantage is that correct disulfide bonds may form because the periplasm provides a more oxidative environment than the cytoplasm. Proteins overexpressed in E. coli are often found in insoluble aggregates, so-called inclusion bodies. These inclusion bodies may be located in the cytosol or in the periplasm; the recovery of biologically active proteins from these inclusion bodies requires a denaturation/refolding process. Many recombinant proteins, including therapeutic proteins, are recovered from inclusion bodies. Alternatively, as will be clear to the skilled person, recombinant strains of bacteria that have been genetically modified so as to secrete a desired protein, and in particular a Nanobody or a polypeptide of the invention, can be used.

Thus, according to one non-limiting embodiment of the invention, the Nanobody or polypeptide of the invention is a Nanobody or polypeptide that has been produced intracellularly and that has been isolated from the host cell, and in particular from a bacterial cell or from an inclusion body in a bacterial cell. According to another non-limiting embodiment of the invention, the Nanobody or polypeptide of the invention is a Nanobody or polypeptide that has been produced extracellularly, and that has been isolated from the medium in which the host cell is cultivated.

Some preferred, but non-limiting promoters for use with these host cells include,

- for expression in E. coli: lac promoter (and derivatives thereof such as the lacUV5 promoter); arabinose promoter; left- (PL) and rightward (PR) promoter of phage lambda; promoter of the trp operon; hybrid lac/trp promoters (tac and trc); T7-promoter (more specifically that of T7-phage gene 10) and other T-phage promoters; promoter of the Tn10 tetracycline resistance gene; engineered variants of the above promoters that include one or more copies of an extraneous regulatory operator sequence;

- for expression in S. cerevisiae: constitutive: ADH1 (alcohol dehydrogenase 1), ENO (enolase), CYC1 (cytochrome c iso-1), GAPDH (glyceraldehydes-3-phosphate dehydrogenase); PGK1 (phosphoglycerate kinase), PYK1 (pyruvate kinase); regulated: GAL1,10,7 (galactose metabolic enzymes), ADH2 (alcohol dehydrogenase 2), PHO5 (acid phosphatase), CUP1 (copper metallothionein); heterologous: CaMV (cauliflower mosaic virus 35S promoter);
- for expression in Pichia pastoris: the AOX1 promoter (alcohol oxidase I)
- for expression in mammalian cells: human cytomegalovirus (hCMV) immediate early enhancer/promoter; human cytomegalovirus (hCMV) immediate early promoter variant that contains two tetracycline operator sequences such that the promoter can be regulated by the Tet repressor; Herpes Simplex Virus thymidine kinase (TK) promoter; Rous Sarcoma Virus long terminal repeat (RSV LTR) enhancer/promoter; elongation factor 1α (hEF-1α) promoter from human, chimpanzee, mouse or rat; the SV40 early promoter; HIV-1 long terminal repeat promoter; β-actin promoter;

Some preferred, but non-limiting vectors for use with these host cells include:

- vectors for expression in mammalian cells: pMAMneo (Clontech), pcDNA3 (Invitrogen), pMC1neo (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593), pBPV-1 (8-2) (ATCC 37110), pdBPV-MMTneo (342-12) (ATCC 37224), pRSVgpt (ATCC37199), pRSVneo (ATCC37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460) and 1ZD35 (ATCC 37565), as well as viral-based expression systems, such as those based on adenovirus;
- vectors for expression in bacterials cells: pET vectors (Novagen) and pQE vectors (Qiagen);
- vectors for expression in yeast or other fungal cells: pYES2 (Invitrogen) and Pichia expression vectors (Invitrogen);
- vectors for expression in insect cells: pBlueBacII (Invitrogen) and other baculovirus vectors
- vectors for expression in plants or plant cells: for example vectors based on cauliflower mosaic virus or tobacco mosaic virus, suitable strains of Agrobacterium, or Ti-plasmid based vectors.

Some preferred, but non-limiting secretory sequences for use with these host cells include:

- for use in bacterial cells such as E. coli: PelB, Bla, OmpA, OmpC, OmpF, OmpT, StII, PhoA, PhoE, MalE, Lpp, LamB, and the like; TAT signal peptide, hemolysin C-terminal secretion signal
- for use in yeast: α-mating factor prepro-sequence, phosphatase (pho1), invertase (Suc), etc.;
- for use in mammalian cells: indigenous signal in case the target protein is of eukaryotic origin; murine Ig κ-chain V-J2-C signal peptide; etc.

Suitable techniques for transforming a host or host cell of the invention will be clear to the skilled person and may depend on the intended host cell/host organism and the genetic construct to be used. Reference is again made to the handbooks and patent applications mentioned above.

After transformation, a step for detecting and selecting those host cells or host organisms that have been succesfully transformed with the nucleotide sequence/genetic construct of the invention may be performed. This may for instance be a selection step based on a selectable marker present in the genetic construct of the invention or a step involving the detection of the amino acid sequence of the invention, e.g. using specific antibodies.

The transformed host cell (which may be in the form or a stable cell line) or host organisms (which may be in the form of a stable mutant line or strain) form further aspects of the present invention.

Preferably, these host cells or host organisms are such that they express, or are (at least) capable of expressing (e.g. under suitable conditions), an amino acid sequence of the invention (and in case of a host organism: in at least one cell, part, tissue or organ thereof). The invention also includes further generations, progeny and/or offspring of the host cell or host organism of the invention, that may for instance be obtained by cell division or by sexual or asexual reproduction.

To produce/obtain expression of the amino acid sequences of the invention, the transformed host cell or transformed host organism may generally be kept, maintained and/or cultured under conditions such that the (desired) amino acid sequence of the invention is expressed/produced. Suitable conditions will be clear to the skilled person and will usually depend upon the host cell/host organism used, as well as on the regulatory elements that control the expression of the (relevant) nucleotide sequence of the invention. Again, reference is made to the handbooks and patent applications mentioned above in the paragraphs on the genetic constructs of the invention.

Generally, suitable conditions may include the use of a suitable medium, the presence of a suitable source of food and/or suitable nutrients, the use of a suitable temperature, and optionally the presence of a suitable inducing factor or compound (e.g. when the nucleotide sequences of the invention are under the control of an inducible promoter); all of which may be selected by the skilled person. Again, under such conditions, the amino acid sequences of the invention may be expressed in a constitutive manner, in a transient manner, or only when suitably induced.

It will also be clear to the skilled person that the amino acid sequence of the invention may (first) be generated in an immature form (as mentioned above), which may then be subjected to post-translational modification, depending on the host cell/host organism used. Also, the amino acid sequence of the invention may be glycosylated, again depending on the host cell/host organism used.

The amino acid sequence of the invention may then be isolated from the host cell/host organism and/or from the medium in which said host cell or host organism was cultivated, using protein isolation and/or purification techniques known per se, such as (preparative) chromatography and/or electrophoresis techniques, differential precipitation techniques, affinity techniques (e.g. using a specific, cleavable amino acid sequence fused with the amino acid sequence of the invention) and/or preparative immunological techniques (i.e. using antibodies against the amino acid sequence to be isolated).

Generally, for pharmaceutical use, the polypeptides of the invention of the inventions may be formulated as a pharmaceutical preparation comprising at least one polypeptide of the invention and at least one pharmaceutically acceptable carrier, diluent or excipient and/or adjuvant, and optionally one or more further pharmaceutically active polypeptides and/or compounds. By means of non-limiting examples, such a formulation may be in a form suitable for oral administration, for parenteral administration (such as by intravenous, intramuscular or subcutaneous injection or intravenous infusion), for topical administration, for administration by inhalation, by a skin patch, by an implant, by a suppository, etc.. Such suitable administration forms - which may be solid, semi-solid or liquid, depending on the manner of administration - as well as methods and carriers for use in the preparation thereof, will be clear to the skilled person, and are further described hereinbelow.

Generally, the Nanobodies and polypeptides of the invention can be formulated and administered in any suitable manner known per se, for which reference is for example made to the general background art cited above (and in particular to WO 04/041862, WO

04/041863, WO 04/041865 and WO 04/041867) as well as to the standard handbooks, such as Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Company, USA (1990) or Remington, the Science and Practice of Pharmacy, 21th Edition, Lippincott Williams and Wilkins (2005).

For example, the Nanobodies and polypeptides of the invention may be formulated and administered in any manner known per se for conventional antibodies and antibody fragments (including ScFv's and diabodies) and other pharmaceutically active proteins. Such formulations and methods for preparing the same will be clear to the skilled person, and for example include preparations suitable for parenteral administration (for example intravenous, intraperitoneal, subcutaneous, intramuscular, intraluminal, intra-arterial or intrathecal administration) or for topical (i.e. transdermal or intradermal) administration.

Preparations for parenteral administration may for example be sterile solutions, suspensions, dispersions or emulsions that are suitable for infusion or injection. Suitable carriers or diluents for such preparations for example include, without limitation, sterile water and aqueous buffers and solutions such as physiological phosphate-buffered saline, Ringer's solutions, dextrose solution, and Hank's solution; water oils; glycerol; ethanol; glycols such as propylene glycol or as well as mineral oils, animal oils and vegetable oils, for example peanut oil, soybean oil, as well as suitable mixtures thereof. Usually, aqueous solutions or suspensions will be preferred.

The Nanobodies and polypeptides of the invention can also be administered using gene therapy methods of delivery. See, *e.g.*, U.S. Patent No. 5,399,346, which is incorporated by reference in its entirety. Using a gene therapy method of delivery, primary cells transfected with the gene encoding a Nanobody or polypeptide of the invention can additionally be transfected with tissue specific promoters to target specific organs, tissue, grafts, tumors, or cells and can additionally be transfected with signal and stabilization sequences for subcellularly localized expression.

Thus, the Nanobodies and polypeptides of the invention may be systemically administered, *e.g.*, orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the Nanobodies and polypeptides of the invention may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups,

wafers, and the like. Such compositions and preparations should contain at least 0.1% of the Nanobody or polypeptide of the invention. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of the Nanobody or polypeptide of the invention in such therapeutically useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the Nanobodies and polypeptides of the invention, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the Nanobodies and polypeptides of the invention may be incorporated into sustained-release preparations and devices.

Preparations and formulations for oral administration may also be provided with an enteric coating that will allow the constructs of the invention to resist the gastric environment and pass into the intestines. More generally, preparations and formulations for oral administration may be suitably formulated for delivery into any desired part of the gastrointestinal tract. In addition, suitable suppositories may be used for delivery into the gastrointestinal tract.

The Nanobodies and polypeptides of the invention may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the Nanobodies and polypeptides of the invention or their salts can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols,

triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form must be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the Nanobodies and polypeptides of the invention in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

For topical administration, the Nanobodies and polypeptides of the invention may be applied in pure form, i.e., when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Useful liquid carriers include water, hydroxyalkyls or glycols or water-alcohol/glycol blends, in which the Nanobodies and polypeptides of the invention can be dissolved or dispersed at effective levels, optionally with the aid of non-

toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

Examples of useful dermatological compositions which can be used to deliver the Nanobodies and polypeptides of the invention to the skin are known to the art; for example, see Jacquet et al. (U.S. Pat. No. 4,608,392), Geria (U.S. Pat. No. 4,992,478), Smith et al. (U.S. Pat. No. 4,559,157) and Wortzman (U.S. Pat. No. 4,820,508).

Useful dosages of the Nanobodies and polypeptides of the invention can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949.

Generally, the concentration of the Nanobodies and polypeptides of the invention in a liquid composition, such as a lotion, will be from about 0.1-25 wt-%, preferably from about 0.5-10 wt-%. The concentration in a semi-solid or solid composition such as a gel or a powder will be about 0.1-5 wt-%, preferably about 0.5-2.5 wt-%.

The amount of the Nanobodies and polypeptides of the invention required for use in treatment will vary not only with the particular Nanobody or polypeptide selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician. Also the dosage of the Nanobodies and polypeptides of the invention varies depending on the target cell, tumor, tissue, graft, or organ.

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, *e.g.*, into a number of discrete loosely spaced administrations; such as multiple inhalations from an insufflator or by application of a plurality of drops into the eye.

An administration regimen could include long-term, daily treatment. By "long-term" is meant at least two weeks and preferably, several weeks, months, or years of duration.

Necessary modifications in this dosage range may be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein. See Remington's Pharmaceutical Sciences (Martin, E.W., ed. 4), Mack Publishing Co., Easton, PA. The dosage can also be adjusted by the individual physician in the event of any complication.

In another aspect, the invention relates to a method for the prevention and/or treatment of at least one TNF-relates disease or disorder as mentioned herein, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of a Nanobody of the invention, of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same.

In the context of the present invention, the term "prevention and/or treatment" not only comprises preventing and/or treating the disease, but also generally comprises preventing the onset of the disease, slowing or reversing the progress of disease, preventing or slowing the onset of one or more symptoms associated with the disease, reducing and/or alleviating one or more symptoms associated with the disease, reducing the severity and/or the duration of the disease and/or of any symptoms associated therewith and/or preventing a further increase in the severity of the disease and/or of any symptoms associated therewith, preventing, reducing or reversing any physiological damage caused by the disease, and generally any pharmacological action that is beneficial to the patient being treated.

The subject to be treated may be any warm-blooded animal, but is in particular a mammal, and more in particular a human being. As will be clear to the skilled person, the subject to be treated will in particular be a person suffering from, or at risk from, the diseases and disorders mentioned herein.

The invention also relates to a method for the prevention and/or treatment of at least one disease or disorder that can be prevented and/or treated by administering a Nanobody or polypeptide of the invention to a patient, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of a Nanobody of the invention, of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same.

More in particular, the invention relates to a method for the prevention and/or treatment of at least one disease or disorder chosen from the group consisting of the diseases and disorders listed herein, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of a Nanobody of the invention, of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same.

In another embodiment, the invention relates to a method for immunotherapy, and in particular for passive immunotherapy, which method comprises administering, to a subject suffering from or at risk of the diseases and disorders mentioned herein, a pharmaceutically active amount of a Nanobody of the invention, of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same.

In the above methods, the Nanobodies and/or polypeptides of the invention and/or the compositions comprising the same can be administered in any suitable manner, depending on the specific pharmaceutical formulation or composition to be used. Thus, the Nanobodies and/or polypeptides of the invention and/or the compositions comprising the same can for example be administered orally, intraperitoneally (e.g. intravenously, subcutaneously, intramuscularly, or via any other route of administration that circumvents the gastrointestinal tract), intranasally, transdermally, topically, by means of a suppository, by inhalation, again depending on the specific pharmaceutical formulation or composition to be used. The clinician will be able to select a suitable route of administration and a suitable pharmaceutical formulation or composition to be used in such administration, depending on the disease or disorder to be prevented or treated and other factorse well known to the clinician.

The Nanobodies and/or polypeptides of the invention and/or the compositions comprising the same are administered according to a regime of treatment that is suitable for preventing and/or treating the disease or disorder to be prevented or treated. The clinician will generally be able to determine a suitable treatment regimen, depending on factors such as the disease or disorder to be prevented or treated, the severity of the disease to be treated and/or the severity of the symptoms thereof, the specific Nanobody or polypeptide of the invention to be used, the specific route of administration and farmaceutical formulation or composition to be used, the age, gender, weight, diet, general condition of the patient, and similar factors well known to the clinician.

Generally, the treatment regimen will comprise the administration of one or more Nanobodies and/or polypeptides of the invention, or of one or more compositions comprising the same, in one or more pharmaceutically effective amounts or doses. The specific amount(s) or doses to administered can be determined by the clinician, again based on the factors cited above.

Generally, for the prevention and/or treatment of the diseases and disorders mentioned herein and depending on the specific disease or disorder to be treated, the potency of the specific Nanobody and polypeptide of the invention to be used, the specific route of

administration and the specific pharmaceutical formulation or composition used, the Nanobodies and polypeptides of the invention will generally be administered in an amount between 1 gram and 0.01 microgram per kg body weight per day, preferably between 0.1 gram and 0.1 microgram per kg body weight per day, such as about 1, 10, 100 or 1000 microgram per kg body weight per day, either continuously (e.g. by infusion), as a single daily dose or as multiple divided doses during the day. The clinician will generally be able to determine a suitable daily dose, depending on the factors mentioned herein. It will also be clear that in specific cases, the clinician may choose to deviate from these amounts, for example on the basis of the factors cited above and his expert judgment. Generally, some guidance on the amounts to be administered can be obtained from the amounts usually administered for comparable conventional antibodies or antibody fragments against the same target administered via essentially the same route, taking into account however differences in affinity/avidity, efficacy, biodistribution, half-life and similar factors well known to the skilled person.

Usually, in the above method, a single Nanobody or polypeptide of the invention will be used. It is however within the scope of the invention to use two or more Nanobodies and/or polypeptides of the invention in combination.

The Nanobodies and polypeptides of the invention may also be used in combination with one or more further pharmaceutically active compounds or principles, i.e. as a combined treatment regimen, which may or may not lead to a synergistic effect. Again, the clinician will be able to select such further compounds or principles, as well as a suitable combined treatment regimen, based on the factors cited above and his expert judgement.

In particular, the Nanobodies and polypeptides of the invention may be used in combination with other pharmaceutically active compounds or principles that are or can be used for the prevention and/or treatment of the diseases and disorders cited herein, as a result of which a synergistic effect may or may not be obtained. Examples of such compounds and principles, as well as routes, methods and pharmaceutical formulations or compositions for administering them will be clear to the clinician.

When two or more substances or principles are to be used as part of a combined treatment regimen, they can be administered via the same route of administration or via different routes of administration, at essentially the same time or at different times (e.g. essentially simultaneously, consecutively, or according to an alternating regime). When the substances or principles are administered to be simultaneously via the same route of

administration, they may be administered as different pharmaceutical formulations or compositions or part of a combined pharmaceutical formulation or composition, as will be clear to the skilled person.

Also, when two or more active substances or principles are to be used as part of a combined treatment regimen, each of the substances or principles may be administered in the same amount and according to the same regimen as used when the compound or principle is used on its own, and such combined use may or may not lead to a synergistic effect. However, when the combined use of the two or more active substances or principles leads to a synergistic effect, it may also be possible to reduce the amount of one, more or all of the substances or principles to be administered, while still achieving the desired therapeutic action. This may for example be useful for avoiding, limiting or reducing any unwanted side-effects that are associated with the use of one or more of the substances or principles when they are used in their usual amounts, while still obtaining the desired pharmaceutical or therapeutic effect.

The effectiveness of the treatment regimen used according to the invention may be determined and/or followed in any manner known per se for the disease or disorder involved, as will be clear to the clinician. The clinician will also be able, where appropriate and or a case-by-case basis, to change or modify a particular treatment regimen, so as to achieve the desired therapeutic effect, to avoid, limit or reduce unwanted side-effects, and/or to achieve an appropriate balance between achieving the desired therapeutic effect on the one hand and avoiding, limiting or reducing undesired side effects on the other hand.

Generally, the treatment regimen will be followed until the desired therapeutic effect is achieved and/or for as long as the desired therapeutic effect is to be maintained. Again, this can be determined by the clinician.

Thus, in a further aspect, the invention relates to a pharmaceutical composition that contains at least one Nanobody of the invention or at least one polypeptide of the invention and at least one suitable carrier (i.e. a carrier suitable for veterinary use), and optionally one or more further active substances.

The invention also relates to the use of a Nanobody of the invention and/or of a polypeptide of the invention in the preparation of a pharmaceutical composition, in particular in the preparation of a pharmaceutical composition for the prevention and/or treatment (including but not limiting to the alleviation of at least one symptom) of a disease or disorder mediated by TNF-alpha and/or associated with TNF-alpha (for example associated with an

abnormal activity of TNF-alpha, abnormal levels of TNF-alpha, abnormal expression of TNF-alpha and/or abnormal sensitivity or response to TNF-alpha, or of one of the biological phenomena associated with TNF-alpha), such as one of the diseases or disorders mentioned above.

The invention also relates to a method for preventing and/or treating (including but not limiting to the alleviation of at least one symptom) of a disease or disorder mediated by TNF-alpha and/or associated with TNF-alpha (for example associated with an abnormal activity of TNF-alpha, abnormal levels of TNF-alpha, abnormal expression of TNF-alpha and/or abnormal sensitivity or response to TNF-alpha, or of one of the biological phenomena associated with TNF-alpha), such as one of the diseases or disorders mentioned above, which method comprises administering to a subject in need thereof a therapeutically active amount of a Nanobody of the invention, of polypeptide of the invention, and/or of a pharmaceutical composition as described above.

The present invention provides polypeptides comprising one or more nanobodies directed towards tumor necrosis factor alpha (TNF-alpha). The present invention further relates to their use in diagnosis and therapy. Such antibodies may have a framework sequence with high homology to the human framework sequences. Compositions comprising antibodies to tumor necrosis factor alpha (TNF-alpha) alone or in combination with other drugs are described.

Tumor necrosis factor alpha (TNF-alpha) is believed to play an important role in various disorders, for example in inflammatory disorders such as rheumatoid arthritis, Crohn's disease, ulcerative colitis and multiple sclerosis. Both TNF-alpha and the receptors (CD120a, CD120b) have been studied in great detail. TNF-alpha in its bioactive form is a trimer and the groove formed by neighboring subunits is important for the cytokine-receptor interaction. Several strategies to antagonize the action of the cytokine have been developed and are currently used to treat various disease states.

A TNF-alpha inhibitor which has sufficient specificity and selectivity to TNF-alpha may be an efficient prophylactic or therapeutic pharmaceutical compound for preventing or treating disorders where TNF-alpha has been implicated as causative agent. Methods of treating toxic shock (EP 486526), tumor regression, inhibition of cytotoxicity (US 6448380, US 6451983, US 6498237), autoimmune disease such as RA and Crohn's disease (EP 663836, US 5672347, US 5656272), graft versus host reaction (US 5672347), bacterial meningitis (EP 585705) by means of an antibody to TNF-alpha have been described.

Yet none of the presently available drugs are completely effective for the treatment of autoimmune disease, and most are limited by severe toxicity. In addition, it is extremely difficult and a lengthy process to develop a new chemical entitiy (NCE) with sufficient potency and selectivity to such target sequence. Antibody-based therapeutics on the other hand have significant potential as drugs because they have exquisite specificity to their target and a low inherent toxicity. In addition, the development time can be reduced considerably when compared to the development of new chemical entities (NCE's). However, conventional antibodies are difficult to raise against multimeric proteins where the receptor-binding domain of the ligand is embedded in a groove, as is the case with TNF-alpha. Heavy chain antibodies described in the invention which are derived from Camelidae, are known to have cavity-binding propensity (WO97/49805; Lauwereys et al, EMBO J. 17, 5312, 1998)). Therefore, such heavy chain antibodies are inherently suited to bind to receptor binding domains of such ligands as TNF. In addition, such antibodies are known to be stable over long periods of time, therefore increasing their shelf-life (Perez et al, Biochemistry, 40, 74, 2001). Furthermore, such heavy chain antibody fragments can be produced 'en-masse' in fermentors using cheap expression systems compared to mammalian cell culture fermentation, such as yeast or other microorganisms (EP 0 698 097).

The use of antibodies derived from sources such as mouse, sheep, goat, rabbit etc., and humanised derivatives thereof as a treatment for conditions which require a modulation of inflammation is problematic for several reasons. Traditional antibodies are not stable at room temperature, and have to be refrigerated for preparation and storage, requiring necessary refrigerated laboratory equipment, storage and transport, which contribute towards time and expense. Refrigeration is sometimes not feasible in developing countries. Furthermore, the manufacture or small-scale production of said antibodies is expensive because the mammalian cellular systems necessary for the expression of intact and active antibodies require high levels of support in terms of time and equipment, and yields are very low. Furthermore the large size of conventional antibodies, would restrict tissue penetration, for example, at the site of inflamed tissue. Furthermore, traditional antibodies have a binding activity which depends upon pH, and hence are unsuitable for use in environments outside the usual physiological pH range such as, for example, in treating gastric bleeding, gastric surgery. Furthermore, traditional antibodies are unstable at low or high pH and hence are not suitable for oral administration. However, it has been demonstrated that camelidae antibodies resist harsh conditions, such as extreme pH, denaturing reagents and high temperatures

(Dumoulin et al, Protein Science 11, 500, 2002), so making them suitable for delivery by oral administration. Furthermore, traditional antibodies have a binding activity, which depends upon temperature, and hence are unsuitable for use in assays or kits performed at temperatures outside biologically active-temperature ranges (e.g. 37 ± 20°C).

Polypeptide therapeutics and in particular antibody-based therapeutics have significant potential as drugs because they have exquisite specificity to their target and a low inherent toxicity. However, it is known by the skilled addressee that an antibody which has been obtained for a therapeutically useful target requires additional modification in order to prepare it for human therapy, so as to avoid an unwanted immunological reaction in a human individual upon administration thereto. The modification process is commonly termed "humanisation". It is known by the skilled artisan that antibodies raised in species, other than in humans, require humanisation to render the antibody therapeutically useful in humans ((1) CDR grafting : Protein Design Labs: US 6180370, US 5693761; Genentech US 6054297; Celltech: 460167, EP 626390, US 5859205; (2) Veneering: Xoma: US 5869619, US 5766886, US 5821123). There is a need for a method for producing antibodies which avoids the requirement for substantial humanisation, or which completely obviates the need for humanisation. There is a need for a new class of antibodies which have defined framework regions or amino acid residues and which can be administered to a human subject without the requirement for substantial humanisation, or the need for humanisation at all.

Another important drawback of conventional antibodies is that they are complex, large molecules and therefore relatively unstable, and they are sensitive to breakdown by proteases. This means that conventional antibody drugs cannot be administered orally, sublingually, topically, nasally, vaginally, rectally or by inhalation because they are not resistant to the low pH at these sites, the action of proteases at these sites and in the blood and/or because of their large size. They have to be administered by injection (intravenously, subcutaneously, etc.) to overcome some of these problems. Administration by injection requires specialist training in order to use a hypodermic syringe or needle correctly and safely. It further requires sterile equipment, a liquid formulation of the therapeutic polypeptide, vial packing of said polypeptide in a sterile and stable form and, of the subject, a suitable site for entry of the needle. Furthermore, subjects commonly experience physical and psychological stress prior to and upon receiving an injection. Therefore, there is need for a method for the delivery of therapeutic polypeptides which avoids the need for injection which

is not only cost/time saving, but which would also be more convenient and more comfortable for the subject.

Nanobody-based therapeutics have significant potential as drugs because they have exquisite specificity to their target and a low inherent toxicity. However, improving further their intrinsic and functional affinity can lead to many benefits for a patient such as reduced dose of therapeutic, faster therapy, and reduced side effects.

One embodiment of the present invention is an anti-TNF-alpha nanobody, which nanobody is preferably as further defined above.

One embodiment of the present invention is an anti-TNF-alpha polypeptide comprising at least one anti-TNF-alpha nanobody, which polypeptide is preferably as further defined above.

Another embodiment of the present invention is an anti-TNF-alpha polypeptide as described above further comprising at least one nanobody directed against a serum protein.

Another embodiment of the present invention is an anti-TNF-alpha polypeptide as described above wherein said serum protein is any of serum albumin, serum immunoglobulins, thyroxine-binding protein, transferrin, or fibrinogen.

Another embodiment of the present invention is an anti-TNF-alpha polypeptide as described above further comprising at least one nanobody selected from the group consisting of anti-IFN-gamma nanobody, anti-TNF-alpha receptor nanobody and anti-IFN-gamma receptor nanobody.

Another embodiment of the present invention is an anti-TNF-alpha polypeptide as described above, wherein the number of nanobodies directed against TNF-alpha is at least two.

Another embodiment of the present invention is an anti-TNF-alpha polypeptide as described above, wherein at least one nanobody is a humanized Camelidae V_{HH}s.

Another embodiment of the present invention is a composition comprising an anti-TNF-alpha polypeptide as described above and at least one nanobody from the group consisting of anti-IFN-gamma nanobody, anti-TNF-alpha receptor nanobody and anti-IFN-gamma receptor nanobody, for simultaneous, separate or sequential administration to a subject.

Another embodiment of the present invention is an anti-TNF-alpha polypeptide as described above, or a composition as described above, wherein said nanobody is an

homologous sequence, a functional portion, or a functional portion of an homologous sequence of the full length nanobody.

Another embodiment of the present invention is an anti-TNF-alpha polypeptide as described above, or a composition as described above, wherein the anti-TNF-alpha polypeptide is an homologous sequence, a functional portion, or a functional portion of an homologous sequence of the full length anti-TNF-alpha polypeptide.

Another embodiment of the present invention is an anti-TNF-alpha polypeptide as described above, or a composition as described above wherein at least one nanobody is a Camelidae V_{HH}.

Another embodiment of the present invention is a nucleic acid encoding an anti-TNF-alpha polypeptide as described above.

Another embodiment of the present invention is a method of identifying an agent that modulates the binding of an anti-TNF-alpha polypeptide as described above, to Tumor Necrosis Factor-alpha comprising the steps of:

(a) contacting an anti-TNF-alpha polypeptide as described above with a target that is Tumor Necrosis Factor alpha, in the presence and absence of a candidate modulator under conditions permitting binding between said polypeptide and target, and

(b) measuring the binding between the polypeptide and target of step (a), wherein a decrease in binding in the presence of said candidate modulator, relative to the binding in the absence of said candidate modulator identified said candidate modulator as an agent that modulates the binding of an anti-TNF-alpha polypeptide as described above and Tumor Necrosis Factor-alpha.

Another embodiment of the present invention is a method of identifying an agent that modulates Tumor Necrosis Factor-alpha-mediated disorders through the binding of an anti-TNF-alpha polypeptide as described above to Tumor Necrosis Factor-alpha comprising:

(a) contacting an anti-TNF-alpha polypeptide as described above with a target that is Tumor Necrosis Factor alpha, in the presence and absence of a candidate modulator under conditions permitting binding between said polypeptide and target, and

(b) measuring the binding between the polypeptide and target of step (a), wherein a decrease in binding in the presence of said candidate modulator, relative to the binding in the absence of said candidate modulator identified, said candidate modulator as an agent that modulates Tumor Necrosis Factor alpha-mediated disorders.

Another embodiment of the present invention is a method of identifying an agent that modulates the binding of Tumor Necrosis Factor alpha to its receptor through the binding of an anti-TNF-alpha polypeptide as described above to Tumor Necrosis Factor-alpha comprising:

(a) contacting an anti-TNF-alpha polypeptide as described above with a target that is Tumor Necrosis Factor-alpha, in the presence and absence of a candidate modulator under conditions permitting binding between said polypeptide and target, and

(b) measuring the binding between the polypeptide and target of step (a), wherein a decrease in binding in the presence of said candidate modulator, relative to the binding in the absence of said candidate modulator identified said candidate modulator as an agent that modulates the binding of Tumor Necrosis Factor-alpha to its receptor.

Another embodiment of the present invention is a kit for screening for agents that modulate Tumor Necrosis Factor-alpha-mediated disorders comprising an anti-TNF-alpha polypeptide as described above and Tumor Necrosis Factor-alpha.

Another embodiment of the present invention is an unknown agent that modulates the binding of an anti-TNF-alpha polypeptide as described above to Tumor Necrosis Factor-alpha, identified according to the method as described above.

Another embodiment of the present invention is an unknown agent that modulates Tumor Necrosis Factor-alpha-mediated disorders, identified according to the methods as described above.

Another embodiment of the present invention is an unknown agent as described above wherein said disorders are one or more of inflammation, rheumatoid arthritis, Crohn's disease, ulcerative colitis, inflammatory bowel syndrome and multiple sclerosis.

Another embodiment of the present invention is an anti-TNF-alpha polypeptide as described above, or a nucleic acid as described above, or a composition as described above, or an agent as described above for treating and/or preventing and/or alleviating disorders relating to inflammatory processes.

Another embodiment of the present invention is a use of an anti-TNF-alpha polypeptide as described above or a nucleic acid as described above, or a composition as described above, or an agent as described above for the preparation of a medicament for treating and/or preventing and/or alleviating disorders relating to inflammatory reactions.

Another embodiment of the present invention is an anti-TNF-alpha polypeptide as described above or a composition as described above, for treating and/or preventing and/or

alleviating disorders susceptible to modulation by a Nanobody or polypeptide of the invention which is able pass through the gastric environment without the substance being inactivated.

Another embodiment of the present invention is an use of an anti-TNF-alpha polypeptide as described above or a composition as described above, for the preparation of a medicament for treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention which is able pass through the gastric environment without the substance being inactivated.

Another embodiment of the present invention is an anti-TNF-alpha polypeptide as described above or a composition as described above, for treating and/or preventing and/or alleviating disorders susceptible to modulation by a Nanobody or polypeptide of the invention delivered to the vaginal and/or rectal tract.

Another embodiment of the present invention is a use of an anti-TNF-alpha polypeptide as described above or a composition as described above, for the preparation of a medicament for treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention delivered to the vaginal and/or rectal tract.

Another embodiment of the present invention is an anti-TNF-alpha polypeptide as described above or a composition as described above, for treating and/or preventing and/or alleviating disorders susceptible to modulation by a Nanobody or polypeptide of the invention delivered to the nose, upper respiratory tract and/or lung.

Another embodiment of the present invention is a use of an anti-TNF-alpha polypeptide as described above or a composition as described above, for the preparation of a medicament for treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention delivered to the nose, upper respiratory tract and/or lung.

Another embodiment of the present invention is an anti-TNF-alpha polypeptide as described above or a composition as described above, for treating and/or preventing and/or alleviating disorders susceptible to modulation by a Nanobody or polypeptide of the invention delivered to the intestinal mucosa, wherein said disorder increases the permeability of the intestinal mucosa.

Another embodiment of the present invention is a use of an anti-TNF-alpha polypeptide as described above or a composition as described above, for the preparation of a

medicament for treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention delivered to the intestinal mucosa, wherein said disorder increases the permeability of the intestinal mucosa.

Another embodiment of the present invention is an anti-TNF-alpha polypeptide as described above or a composition as described above, for treating and/or preventing and/or alleviating disorders susceptible to modulation by a Nanobody or polypeptide of the invention which is able pass through the tissues beneath the tongue effectively.

Another embodiment of the present invention is a use of an anti-TNF-alpha polypeptide as described above or a composition as described above, for the preparation of a medicament for treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention which is able pass through the tissues beneath the tongue effectively.

Another embodiment of the present invention is an anti-TNF-alpha polypeptide as described above or a composition as described above, for treating and/or preventing and/or alleviating disorders susceptible to modulation by a Nanobody or polypeptide of the invention which is able pass through the skin effectively.

Another embodiment of the present invention is a use of an anti-TNF-alpha polypeptide as described above or a composition as described above, for the preparation of a medicament for treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention which is able pass through the skin effectively.

Another embodiment of the present invention is a method as described above, a kit as described above, a nucleic acid or agent as described above, use of a nucleic acid or agent as described above, a composition as described above, use of a composition as described above, an anti-TNF-alpha polypeptide as described above, use of an anti-TNF-alpha polypeptide as described above wherein said disorders are any of inflammation, rheumatoid arthritis, COPD, asthma, Crohn's disease, ulcerative colitis, inflammatory bowel syndrome, multiple sclerosis, Addison's disease, Autoimmune hepatitis, Autoimmune parotitis, Diabetes Type I, Epididymitis, Glomerulonephritis, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, Hemolytic anemia, Systemic lupus erythematosus, Male infertility, Multiple sclerosis, Myasthenia Gravis, Pemphigus, Psoriasis, Rheumatic fever, Rheumatoid arthritis, Sarcoidosis, Scleroderma, Sjogren's syndrome, Spondyloarthropathies, Thyroiditis, and Vasculitis.

Another embodiment of the present invention is a composition comprising a nucleic acid or agent as described above, an anti-TNF-alpha polypeptide as described above, or a composition as described above, and a suitable pharmaceutical vehicle.

Another embodiment of the present invention is a method of diagnosing a disorder characterised by the dysfunction of Tumor Necrosis Factor-alpha comprising:

(a) contacting a sample with an anti-TNF-alpha polypeptide as described above,

(b) detecting binding of said polypeptide to said sample, and

(c) comparing the binding detected in step (b) with a standard, wherein a difference in binding relative to said sample is diagnostic of a disorder characterised by dysfunction of Tumor Necrosis Factor-alpha.

Another embodiment of the present invention is a kit for screening for a disorder as cited above, using a method as described above.

Another embodiment of the present invention is a kit for screening for a disorder as cited above comprising an isolated anti-TNF-alpha polypeptide as described above.

Another embodiment of the present invention is a use of an anti-TNF-alpha polypeptide as described above for the purification of said Tumor Necrosis Factor-alpha.

Another embodiment of the present invention is a use of an anti-TNF-alpha polypeptide as described above for inhibiting the interaction between Tumor Necrosis Factor-alpha and one or more Tumor Necrosis Factor-alpha receptors.

Another embodiment of the present invention is a method for producing an anti-TNF-alpha polypeptide as described above comprising the steps of:

(a) obtaining double stranded DNA encoding a Camelidae V_{HH} directed to Tumor Necrosis Factor alpha,

(b) cloning and expressing the DNA selected in step (b).

Another embodiment of the present invention is a method of producing an anti-TNF-alpha polypeptide as described above comprising:

(a) culturing host cells comprising nucleic acid capable of encoding an anti-TNF-alpha polypeptide as described above, under conditions allowing the expression of the polypeptide, and,

(b) recovering the produced polypeptide from the culture.

Another embodiment of the present invention is a method as described above, wherein said host cells are bacterial or yeast.

Another embodiment of the present invention is a kit for screening for any of inflammation, rheumatoid arthritis, Crohn's disease, ulcerative colitis, inflammatory bowel syndrome or multiple sclerosis comprising an anti-TNF-alpha polypeptide as described above.

V_{HH}s, according to the present invention, and as known to the skilled addressee are heavy chain variable domains derived from immunoglobulins naturally devoid of light chains such as those derived from Camelidae as described in WO 94/04678 (and referred to hereinafter as V_{HH} domains or nanobodies). V_{HH} molecules are about 10x smaller than IgG molecules. They are single polypeptides and very stable, resisting extreme pH and temperature conditions. Moreover, they are resistant to the action of proteases which is not the case for conventional antibodies. Furthermore, in vitro expression of V_{HH}s produces high yield, properly folded functional V_{HH}s. In addition, antibodies generated in Camelids will recognize epitopes other than those recognised by antibodies generated in vitro through the use of antibody libraries or via immunisation of mammals other than Camelids (WO 9749805). As such, anti-TNF-alpha V_{HH}'s may interact more efficiently with TNF-alpha than conventional antibodies, thereby blocking its interaction with the TNF-alpha receptor more efficiently.

TNF-alpha is also a fragment of TNF-alpha, capable of eliciting an immune response. TNF-alpha is also a fragment of TNF-alpha, capable of binding to a nanobody raised against the full length TNF-alpha.

A nanobody directed against TNF-alpha means nanobody that it is capable of binding to TNF-alpha with an affinity of better than 10^{-6} M.

One embodiment of the present invention is an anti-TNF polypeptide, wherein the nanobodies comprise Camelidae V_{HH} directed against TNF-alpha.

The one or more nanobodies of the anti-TNF polypeptide which are directed against a TNF-alpha may be of the same sequence. Alternatively they may not all have the same sequence. It is within the scope of the invention that an anti-TNF polypeptide comprises anti-TNF-alpha nanobodies which do not all share the same sequence, but which are directed against the same target, one or more antigens thereof.

The present invention further relates to an anti-TNF-alpha polypeptide, wherein said nanobody is a V_{HH} directed against TNF-alpha, wherein the V_{HH} belongs to a class having human-like sequences. The class is characterised in that the V_{HH}s carry an amino acid from the group consisting of glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine,

tyrosine, tryptophan, methionine, serine, threonine, asparagine, or glutamine at position 45, such as, for example, L45 and a tryptophan at position 103, according to the Kabat numbering. Another human-like class of Camelidae nanobodies have been described in WO03035694 and contain the hydrophobic FR2 residues typically found in conventional antibodies of human origin or from other species, but compensating this loss in hydrophilicity by the charged arginine residue on position 103 that substitutes the conserved tryptophan residue present in VH from double-chain antibodies. As such, peptides belonging to these two classes show a high amino acid sequence homology to human VH framework regions and said peptides might be administered to a human directly without expectation of an unwanted immune response therefrom, and without the burden of further humanisation. The invention also relates to nucleic acids capable of encoding said polypeptides.

Any of the V_{HH}s as used by the invention may be of the traditional class or of the classes of human-like Camelidae antibodies. Said antibodies may be directed against whole TNF-alpha or a fragment thereof, or a fragment of a homologous sequence thereof. These polypeptides include the full length Camelidae antibodies, namely Fc and V_{HH} domains, chimeric versions of heavy chain Camelidae antibodies with a human Fc domain or V_{HH}'s by themselves or derived fragments.

Anti-serum albumin V_{HH}'s may interact in a more efficient way with serum albumin than conventional antibodies which is known to be a carrier protein. As a carrier protein some of the epitopes of serum albumin may be inaccessible by bound proteins, peptides and small chemical compounds. Since V_{HH}'s are known to bind into 'unusual' or non-conventional epitopes such as cavities (WO 97/49805), the affinity of such V_{HH}'s to circulating albumin may be increased.

The present invention also relates to the finding that an anti-TNF polypeptide as described herein further comprising one or more nanobodies directed against one or more serum proteins of a subject, surprisingly has significantly prolonged half-life in the circulation of said subject compared with the half-life of the anti-TNF-alpha nanobody when not part of said construct. Furthermore, the said polypeptides were found to exhibit the same favourable properties of nanobodies such as high stability remaining intact in mice, extreme pH resistance, high temperature stability and high target affinity.

The serum protein may be any suitable protein found in the serum of subject. In one aspect of the invention, the serum protein is serum albumin, serum immunoglobulins, thyroxine-binding protein, transferrin, or fibrinogen. Depending on the intended use such as

the required half-life for effective treatment and/or compartimentalisation of the target antigen, the V_{HH}-partner can be directed to one of the above serum proteins.

According to a specific, but non-limiting aspect of the invention, the Nanobody against human serum albumin consists of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), in which:

(iv) CDR1 is an amino acid sequence chosen from the group consisting of:

SFGMS	[SEQ ID NO: 36]
LNLMG	[SEQ ID NO: 37]
INLLG	[SEQ ID NO: 38]
NYWMY;	[SEQ ID NO: 39]

and/or from the group consisting of amino acid sequences that have 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequences;

and in which:

(v) CDR2 is an amino acid sequence chosen from the group consisting of:

SISGSGSDTLYADSVKG	[SEQ ID NO: 40]
TITVGDSTNYADSVKG	[SEQ ID NO: 41]
TITVGDSTSYADSVKG	[SEQ ID NO: 42]
SINGRGDDTRYADSVKG	[SEQ ID NO: 43]
AISADSSTKNYADSVKG	[SEQ ID NO: 44]
AISADSSDKRYADSVKG	[SEQ ID NO: 45]
RISTGGGYSYYADSVKG	[SEQ ID NO: 46]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the above amino acid sequences; in which

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequences;

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequences;

and in which:

(vi) CDR3 is an amino acid sequence chosen from the group consisting of:

DREAQVDTLDFDY [SEQ ID NO: 47]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the above amino acid sequences; in which

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequences;

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequences;

or from the group consisting of:

GGSLSR [SEQ ID NO: 48]

RRTWHSEL [SEQ ID NO: 49]

GRSVSRS [SEQ ID NO: 50]

GRGSP [SEQ ID NO: 51]

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequences.

In another aspect, the invention relates to a Nanobody against human serum albumin, which consist of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), which is chosen from the group consisting of domain antibodies and/or single domain antibodies with the one of the following combinations of CDR1, CDR2 and CDR3, respectively:

- **CDR1:** SFGMS; **CDR2:** SISGSGSDTLYADSVKG; **CDR3:** GGSLSR;
- **CDR1:** LNLMG; **CDR2:** TITVGDSTNYADSVKG; **CDR3:** RRTWHSEL;
- **CDR1:** INLLG; **CDR2:** TITVGDSTSYADSVKG; **CDR3:** RRTWHSEL;
- **CDR1:** SFGMS; **CDR2:** SINGRGDDTRYADSVKG; **CDR3:** GRSVSRS;
- **CDR1:** SFGMS; **CDR2:** AISADSSDKRYADSVKG; **CDR3:** GRGSP;
- **CDR1:** SFGMS; **CDR2:** AISADSSDKRYADSVKG; **CDR3:** GRGSP;
- **CDR1:** NYWMY; **CDR2:** RISTGGGYSYYADSVKG; **CDR3:** DREAQVDTLDFDY.

In the Nanobodies of the invention that comprise the combinations of CDR's mentioned above, each CDR can be replaced by a CDR chosen from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with the mentioned CDR's; in which

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequences;

and/or chosen from the group consisting of amino acid sequences that have 3, 2 or only 1 (as indicated in the preceding paragraph) "amino acid difference(s)" (as defined herein) with the mentioned CDR(s) one of the above amino acid sequences, in which:

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequences.

However, of the Nanobodies of the invention that comprise the combinations of CDR's mentioned above, Nanobodies comprising one or more of the CDR's listed above are particularly preferred; Nanobodies comprising two or more of the CDR's listed above are more particularly preferred; and Nanobodies comprising three of the CDR's listed above are most particularly preferred.

In these Nanobodies against human serum albumin, the Framework regions FR1 to FR4 are preferably as defined hereinabove for the Nanobodies of the invention.

Particularly preferred Nanobodies against human serum albumin are chosen from the group consisting of SEQ ID NO's: 61 to 67, SEQ ID NO's 87 to 89 and SEQ ID NO's 100-104. The preferred combinations of CDR's and framework regions present in these Nanobodies are also listed in Table II

Table II: Preferred combination of Framework sequences and CDR's in Nanobodies against human serum albumin.

Clone	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
PMP6A8 (ALB2)	368	AVQLVESGGGLVQGGG SLRLACAASERIFD	375	LNLMG	382	WYRQGPGN ERELVA	389	TCITVGDSTNYA DSVKG	396	RFTISMDYTKQTVYLHMN SLRPEDTGLYYCKI	403	RRTWHSEL	410	WGQGTQV TVSS
PMP6B4	369	EVQLVESGGGLVQEGG SLRLACAASERIWD	376	INLLG	383	WYRQGPGN ERELVA	390	TITVGDSTSYAD SVKG	397	RFTISRDYDKNTLYLQMN SLRPEDTGLYYCKI	404	RRTWHSEL	411	WGQGTQV TVSS
PMP6A6 (ALB1)	370	AVQLVESGGGLVQPGN SLRLSCAASGFTFR	377	SFGMS	384	WVRQAPGK EPEWVS	391	SISGSGSDTLYA DSVKG	398	RFTISRDNAKTTLYLQMN SLKPEDTAVYYCTI	405	GGSLSR	412	SSQGTQV TVSS
PMP6C1	371	AVQLVDSGGGLVQPGG SLRLSCAASGFSFG	378	SFGMS	385	WVRQYPGK EPEWVS	392	SINGRGDDTRYA DSVKG	399	RFSISRDNAKNTLYLQMN SLKPEDTAEYYCTI	406	GRSVSRS	413	RTQGTQV TVSS
PMP6G8	372	AVQLVESGGGLVQPGG SLRLTCTASGFTFR	379	SFGMS	386	WVRQAPGK DQEWVS	393	AISADSSTKNYA DSVKG	400	RFTISRDNAKKMLYLEMN SLKPEDTAVYYCVI	407	GRGSP	414	SSPGTQV TVSS
PMP6A5	373	QVQLAESGGGLVQPGG SLRLTCTASGFTFG	380	SFGMS	387	WVRQAPGE GLEWVS	394	AISADSSDKRYA DSVKG	401	RFTISRDNAKKMLYLEMN SLKSEDTAVYYCVI	408	GRGSP	415	ASQGTQV TVSS
PMP6G7	374	QVQLVESGGGLVQPGG SLRLSCAASGFTFS	381	NYWMY	388	WVRVAPGK GLERIS	395	RDISTGGGYSYY ADSVKG	402	RFTISRDNAKNTLYLQMN SLKPEDTALYYCAK	409	DREAQVDTLD FDY	416	RGQGTQV TVSS

107

Another aspect of the invention is an anti-TNF-alpha polypeptide as disclosed herein further comprising at least one polypeptide selected from the group consisting of an anti-IFN-gamma polypeptide, an anti-TNF-alpha receptor polypeptide and anti-IFN-gamma receptor polypeptide.

According to one aspect of the invention, a nanobody is directed against TNF-alpha receptor. Said nanobody may be a Camelidae V_{HH}.

According to one aspect of the invention, a nanobody is directed against IFN-gamma receptor. Said nanobody may be a Camelidae V_{HH}.

Another aspect of the invention is a method of treating an autoimmune disease or condition as cited herein, comprising administering to a patient an effective amount of an anti-TNF-alpha polypeptide further comprising a least one polypeptide selected from the group consisting of anti-IFN-gamma polypeptide, anti-TNF-alpha receptor polypeptide and anti-IFN-gamma receptor polypeptide, such polypeptides joined to each other as described below.

Such multi-specific constructs may have improved potency as inflammatory therapeutic compound over mono-specific constructs.

One aspect of the invention is a composition comprising an anti-TNF-alpha polypeptide as disclosed herein and at least one polypeptide selected from the group consisting of anti-IFN-gamma polypeptide, anti-TNF-alpha receptor polypeptide and anti-IFN-gamma receptor polypeptide, for simultaneous, separate or sequential administration to a subject.

One aspect of the invention is a method for treating autoimmune disease comprising administering to an individual an effective amount of an anti-TNF-alpha polypeptide and a least one polypeptide selected from the group consisting of anti-IFN-gamma polypeptide, anti-TNF-alpha receptor polypeptide and anti-IFN-gamma receptor polypeptide, simultaneously, separately or sequentially.

Another aspect of the invention is a kit containing an anti-TNF-alpha polypeptide and a least one polypeptide selected from the group consisting of anti-IFN-gamma polypeptide, anti-TNF-alpha receptor polypeptide and anti-IFN-gamma receptor polypeptide for simultaneous, separate or sequential administration to a subject. It is an aspect of the invention that the kit may be used according to the invention. It is an aspect of the invention that the kit may be used to treat the diseases as cited herein.

By simultaneous administration means the polypeptides are administered to a subject at the same time. For example, as a mixture of the polypeptides or a composition comprising said polypeptides. Examples include, but are not limited to a solution administered intraveneously, a tablet, liquid, topical cream, etc., wherein each preparation comprises the polypeptides of interest.

By separate administration means the polypeptides are administered to a subject at the same time or substantially the same time. The polypeptides are present in the kit as separate, unmixed preparations. For example, the different polypeptides may be present in the kit as individual tablets. The tablets may be administered to the subject by swallowing both tablets at the same time, or one tablet directly following the other.

By sequential administration means the polypeptides are administered to a subject sequentially. The polypeptides are present in the kit as separate, unmixed preparations. There is a time interval between doses. For example, one polypeptide might be administered up to 336, 312, 288, 264, 240, 216, 192, 168, 144, 120, 96, 72, 48, 24, 20, 16, 12, 8, 4, 2, 1, or 0.5 hours after the other component.

In sequential administration, one polypeptide may be administered once, or any number of times and in various doses before and/or after administration of another polypeptide. Sequential administration may be combined with simultaneous or sequential administration.

The medical uses of the anti-TNF-alpha polypeptide described below, also apply to the composition comprising an anti-TNF-alpha polypeptide as disclosed herein and at least one polypeptide selected from the group consisting of anti-IFN-gamma polypeptide, anti-TNF-alpha receptor polypeptide and anti-IFN-gamma receptor polypeptide, for simultaneous, separate or sequential administration to a subject as disclosed here above.

According to one aspect of the invention, an anti-IFN-gamma polypeptide anti-TNF-alpha a nanobody directed against IFN-gamma. Said nanobody may be a Camelidae V_{HH}.

According to one aspect of the invention, anti-TNF-alpha a nanobody directed against TNF-alpha receptor. Said nanobody may be a Camelidae V_{HH}.

According to one aspect of the invention, an anti-IFN-gamma receptor polypeptide anti-TNF-alpha a nanobody directed against IFN-gamma receptor. Said nanobody may be a Camelidae V_{HH}.

Another embodiment of the present invention is an anti-TNF-alpha polypeptide as disclosed herein, wherein the number of nanobodies directed against TNF-alpha is two or

more. Such multivalent anti-TNF-alpha polypeptides have the advantage of unusually high functional affinity for the target, displaying much higher than expected inhibitory properties compared to their monovalent counterparts.

The multivalent anti-TNF-alpha polypeptides have functional affinities that are several orders of magnitude higher than the monovalent parent anti-TNF-alpha polypeptides. The inventors have found that the functional affinities of these multivalent polypeptides are much higher than those reported in the prior art for bivalent and multivalent antibodies. Surprisingly, anti-TNF-alpha polypeptides of the present invention linked to each other directly or via a short linker sequence show the high functional affinities expected theoretically with multivalent conventional four-chain antibodies.

The inventors have found that such large increased functional activities can be detected preferably with antigens composed of multidomain and multimeric proteins, either in straight binding assays or in functional assays, e.g. cytotoxicity assays.

The nanobodies may be joined to form any of the polypeptides disclosed herein comprising more than one nanobody using methods known in the art or any future method. For example, they may be fused by chemical cross-linking by reacting amino acid residues with an organic derivatising agent such as described by Blattler et al, Biochemistry 24,1517-1524; EP294703. Alternatively, the nanobody may be fused genetically at the DNA level i.e. a polynucleotide construct formed which encodes the complete polypeptide construct comprising one or more anti-target nanobodies and one or more anti-serum protein nanobodies. A method for producing bivalent or multivalent V_{HH} polypeptide constructs is disclosed in PCT patent application WO 96/34103. One way of joining multiple nanobodies is via the genetic route by linking nanobody coding sequences either directly or via a peptide linker. For example, the C-terminal end of the first nanobody may be linked to the N-terminal end of the next nanobody. This linking mode can be extended in order to link additional nanobodies for the construction and production of tri-, tetra-, etc. functional constructs.

According to one aspect of the present invention, the nanobodies are linked to each other directly, without use of a linker. Contrary to joining bulky conventional antibodies where a linker sequence is needed to retain binding activity in the two subunits, polypeptides of the invention can be linked directly thereby avoiding potential problems of the linker sequence, such as antigenicity when administered to a human subject, instability of the linker sequence leading to dissociation of the subunits.

According to another aspect of the present invention, the nanobodies are linked to each other via a peptide linker sequence. Such linker sequence may be a naturally occurring sequence or a non-naturally occurring sequence. The linker sequence is expected to be non-immunogenic in the subject to which the anti-TNF-alpha polypeptide is administered. The linker sequence may provide sufficient flexibility to the multivalent anti-TNF-alpha polypeptide, at the same time being resistant to proteolytic degradation. A non-limiting example of a linker sequences is one that can be derived from the hinge region of V_{HH}s described in WO 96/34103.

According to another aspect of the invention, multivalent nanobodies comprising more than two nanobodies can be linked to each other either directly or via a linker sequence. Such constructs are difficult to produce with conventional antibodies and due to steric hindrance of the bulky subunits, functionality will be lost or greatly diminished rather than increased considerably as seen with V_{HH}'s of the invention compared to the monovalent construct.

The polypeptide constructs disclosed herein may be made by the skilled artisan according to methods known in the art or any future method. For example, V_{HH}s may be obtained using methods known in the art such as by immunising a camel and obtaining hybridomas therefrom, or by cloning a library of nanobodies using molecular biology techniques known in the art and subsequent selection by using phage display.

According to an aspect of the invention an anti-TNF-alpha polypeptide may be a homologous sequence of a full-length anti-TNF-alpha polypeptide. According to another aspect of the invention, an anti-TNF-alpha polypeptide may be a functional portion of a full-length anti-TNF-alpha polypeptide. According to another aspect of the invention, an anti-TNF-alpha polypeptide may be a homologous sequence of a full-length anti-TNF-alpha polypeptide. According to another aspect of the invention, an anti-TNF-alpha polypeptide may be a functional portion of a homologous sequence of a full-length anti-TNF-alpha polypeptide. According to an aspect of the invention an anti-TNF-alpha polypeptide may comprise a sequence of an anti-TNF-alpha polypeptide.

According to an aspect of the invention a nanobody used to form an anti-TNF-alpha polypeptide may be a complete nanobody (e.g. a V_{HH}) or a homologous sequence thereof. According to another aspect of the invention, a nanobody used to form the polypeptide construct may be a functional portion of a complete nanobody. According to another aspect of the invention, a nanobody used to form the polypeptide construct may be a homologous

sequence of a complete nanobody. According to another aspect of the invention, a nanobody used to form the polypeptide construct may be a functional portion of a homologous sequence of a complete nanobody.

As used herein, an homologous sequence of the present invention may comprise additions, deletions or substitutions of one or more amino acids, which do not substantially alter the functional characteristics of the polypeptides of the invention. The number of amino acid deletions or substitutions is preferably up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69 or 70 amino acids.

A homologous sequence according to the present invention may a polypeptide modified by the addition, deletion or substitution of amino acids, said modification not substantially altering the functional characteristics compared with the unmodified polypeptide.

A homologous sequence according to the present invention may be a polypeptide modified by the addition, deletion or substitution of amino acids, said modification not substantially altering the functional characteristics compared with the unmodified polypeptide.

A homologous sequence according to the present invention may be a sequence which exists in other Camelidae species such as, for example, camel, dromedary, llama, alpaca, guanaco etc.

Where homologous sequence indicates sequence identity, it means a sequence which presents a high sequence identity (more than 70%, 75%, 80%, 85%, 90%, 95% or 98% sequence identity) with the parent sequence and is preferably characterised by similar properties of the parent sequence, namely affinity, said identity calculated using known methods.

Alternatively, an homologous sequence may also be any amino acid sequence resulting from allowed substitutions at any number of positions of the parent sequence according to the formula below:

Ser substituted by Ser, Thr, Gly, and Asn;
Arg substituted by one of Arg, His, Gln, Lys, and Glu;
Leu substituted by one of Leu, Ile, Phe, Tyr, Met, and Val;
Pro substituted by one of Pro, Gly, Ala, and Thr;

Thr substituted by one of Thr, Pro, Ser, Ala, Gly, His, and Gln;
Ala substituted by one of Ala, Gly, Thr, and Pro;
Val substituted by one of Val, Met, Tyr, Phe, Ile, and Leu;
Gly substituted by one of Gly, Ala, Thr, Pro, and Ser;
Ile substituted by one of Ile, Met, Tyr, Phe, Val, and Leu;
Phe substituted by one of Phe, Trp, Met, Tyr, Ile, Val, and Leu;
Tyr substituted by one of Tyr, Trp, Met, Phe, Ile, Val, and Leu;
His substituted by one of His, Glu, Lys, Gln, Thr, and Arg;
Gln substituted by one of Gln, Glu, Lys, Asn, His, Thr, and Arg;
Asn substituted by one of Asn, Glu, Asp, Gln, and Ser;
Lys substituted by one of Lys, Glu, Gln, His, and Arg;
Asp substituted by one of Asp, Glu, and Asn;
Glu substituted by one of Glu, Asp, Lys, Asn, Gln, His, and Arg;
Met substituted by one of Met, Phe, Ile, Val, Leu, and Tyr.

A homologous nucleotide sequence according to the present invention may refer to nucleotide sequences of more than 50, 100, 200, 300, 400, 500, 600, 800 or 1000 nucleotides able to hybridize to the reverse-complement of the nucleotide sequence capable of encoding the patent sequence, under stringent hybridisation conditions (such as the ones described by Sambrook et al., Molecular Cloning, Laboratory Manuel, Cold Spring, Harbor Laboratory press, New York).

As used herein, a functional portion refers to a sequence of a nanobody that is of sufficient size such that the interaction of interest is maintained with affinity of 1×10^{-6} M or better.

Alternatively, a functional portion comprises a partial deletion of the complete amino acid sequence and still maintains the binding site(s) and protein domain(s) necessary for the binding of and interaction with the target.

As used herein, a functional portion refers to less than 100% of the complete sequence (e.g., 99%, 90%, 80%, 70%, 60% 50%, 40%, 30%, 20%, 10%, 5%, 1% etc.), but comprises 5 or more amino acids or 15 or more nucleotides.

Targets as mentioned herein such as TNF-alpha, TNF-alpha receptor, serum proteins (e.g. serum albumin, serum immunoglobulins, thyroxine-binding protein, transferrin, fibrinogen) and IFN-gamma, IFN-gamma receptor may be fragments of said targets. Thus a target is also a fragment of said target, capable of eliciting an immune response. A target is

also a fragment of said target, capable of binding to a nanobody raised against the full length target.

A fragment as used herein refers to less than 100% of the sequence (e.g., 99%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% etc.), but comprising 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more amino acids. A fragment is of sufficient length such that the interaction of interest is maintained with affinity of 1×10^{-6} M or better.

A fragment as used herein also refers to optional insertions, deletions and substitutions of one or more amino acids which do not substantially alter the ability of the target to bind to a nanobody raised against the wild-type target. The number of amino acid insertions deletions or substitutions is preferably up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69 or 70 amino acids.

A homologous sequence of the present invention may include an anti-TNF-alpha polypeptide which has been humanised. The humanisation of antibodies of the new class of V_{HH}s would further reduce the possibility of unwanted immunological reaction in a human individual upon administration.

One embodiment of the present invention relates to a method for preparing modified polypeptides based upon llama antibodies by determining the amino acid residues of the antibody variable domain (V_{HH}) which may be modified without diminishing the native affinity of the domain for antigen and while reducing its immunogenicity with respect to a heterologous species; the use of V_{HH}s having modifications at the identified residues which are useful for administration to heterologous species; and to the V_{HH} so modified.

More specifically, the invention relates to the preparation of modified V_{HH}s, which are modified for administration to humans, the resulting V_{HH} themselves, and the use of such "humanized" V_{HH}s in the treatment of diseases in humans. By humanised is meant mutated so that immunogenicity upon administration in human patients is minor or nonexistent. Humanising a polypeptide, according to the present invention, comprises a step of replacing one or more of the Camelidae amino acids by their human counterpart as found in the human consensus sequence, without that polypeptide losing its typical character, i.e. the humanisation does not significantly affect the antigen binding capacity of the resulting polypeptide. Such methods are known by the skilled addressee.

Humanization of Camelidae nanobodies requires the introduction and mutagenesis of a limited amount of amino acids in a single polypeptide chain. This is in contrast to humanization of scFv, Fab, (Fab)2 and IgG, which requires the introduction of amino acid changes in two chains, the light and the heavy chain and the preservation of the assembly of both chains.

As described in WO 04/041862, an anti-TNF nanobody can be humanized. Humanization may for example involve mutagenesis of residues in FR1 at position 1 and 5 which were introduced by the primer used for repertoire cloning and do not occur naturally in the llama sequence. Mutagenesis of those residues did not result in loss of binding and/or inhibition activity. Humanization may also involve mutagenesis of residues in FR3 at position 74, 76, 83, 84, 93. Mutagenesis of those residues did not result in a dramatic loss of binding and/or inhibition activity. Combining the mutations of FR1 and FR3 therefore did not affect the binding and/or inhibition activity. Humanization may also involve mutagenesis of residues in FR4 at position 108. Mutagenesis of Q108L resulted in lower production level in Escherichia coli. Position 108 is solvent exposed in camelid V_{HH}, while in human antibodies this position is buried at the VH-VL interface (Spinelli, 1996; Nieba, 1997). In isolated VHs position 108 is solvent exposed. The introduction of a non-polar hydrophobic Leu instead of polar uncharged Gln can have a drastic effect on the intrinsic folding/stability of the molecule. Also, replacement of the hydrophilic residues by human hydrophobic residues at positions 44 and 45 (E44G and R45L), did not have an effect on binding and/or inhibition. However, loss of binding and/or inhibition activity was observed when F37V and F47W were introduced. Modeling data confirmed the critical residue 37 to preserve the integrity of the CDR3 loop conformation and hence on activity (all numbering according to the Kabat).

According to one embodiment of the present invention, humanization involves replacing of any of the following residues either alone or in combination:

- FR1 position 1, 5, 28 and 30,
- the hallmark amino acid at position 44 and 45 in FR2,
- FR3 residues 74, 75, 76, 83, 84, 93 and 94 ,
- and positions 103, 104, 108 and 111 in FR4 ;
- numbering according to the Kabat numbering.

One embodiment of the present invention is an anti-TNF-alpha polypeptide, or a nucleic acid capable of encoding said polypeptide for use in treating, preventing and/or alleviating the symptoms of disorders relating to inflammatory processes. TNF-alpha is

involved in inflammatory processes, and the blocking of TNF-alpha action can have an anti-inflammatory effect, which is highly desirable in certain disease states such as, for example, Crohn's disease. The Examples demonstrate V_{HH}s according to the invention which bind TNF-alpha and moreover, block its binding to the TNF-alpha receptor.

The anti-TNF-alpha polypeptides of the present invention are applicable to autoimmune diseases, such as Addison's disease (adrenal), Autoimmune diseases of the ear (ear), Autoimmune diseases of the eye (eye), Autoimmune hepatitis (liver), Autoimmune parotitis (parotid glands), Crohn's disease (intestine), Diabetes Type I (pancreas), Epididymitis (epididymis), Glomerulonephritis (kidneys), Graves' disease (thyroid), Guillain-Barre syndrome (nerve cells), Hashimoto's disease (thyroid), Hemolytic anemia (red blood cells), Systemic lupus erythematosus (multiple tissues), Male infertility (sperm), Multiple sclerosis (nerve cells), Myasthenia Gravis (neuromuscular junction), Pemphigus (primarily skin), Psoriasis (skin), Rheumatic fever (heart and joints), Rheumatoid arthritis (joint lining), Sarcoidosis (multiple tissues and organs), Scleroderma (skin and connective tissues), Sjogren's syndrome (exocrine glands, and other tissues), Spondyloarthropathies (axial skeleton, and other tissues), Thyroiditis (thyroid), Vasculitis (blood vessels).
Within parenthesis is the tissue affected by the disease. This listing of autoimmune diseases is intended to be exemplary rather than inclusive.

Autoimmune conditions for which the anti-TNF-alpha polypeptides of the present invention is applicable include, for example, AIDS, atopic allergy, bronchial asthma, eczema, leprosy, schizophrenia, inherited depression, transplantation of tissues and organs, chronic fatigue syndrome, Alzheimer's disease, Parkinson's disease, myocardial infarction, stroke, autism, epilepsy, Arthus's phenomenon, anaphylaxis, and alcohol and drug addiction. In the above-identified autoimmune conditions, the tissue affected is the primary target, in other cases it is the secondary target. These conditions are partly or mostly autoimmune syndromes. Therefore, in treating them, it is possible to use the same methods, or aspects of the same methods that are herein disclosed, sometimes in combination with other methods.

Another embodiment of the present invention is a use of an anti-TNF-alpha polypeptide according to the invention, or a nucleic acid capable of encoding said polypeptide for the preparation of a medicament for treating a disorder relating to inflammatory processes. Examples of disorders include rheumatoid arthritis, Crohn's disease, ulcerative colitis, inflammatory bowel syndrome and multiple sclerosis.

Polypeptides and nucleic acids according to the present invention may be administered to a subject by conventional routes, such as intravenously. However, a special property of the anti-TNF-alpha polypeptides of the invention is that they penetrate barriers such as tissue membranes and/or tumours and act locally thereon, and they are sufficiently stable to withstand extreme environments such as in the stomach. Therefore, another aspect of the present invention relates to the delivery of anti-TNF-alpha polypeptides.

When the Nanobodies and/or polypeptides of the invention are used for, or are intended for use in, the prevention or treatment of diseases and disorders of the gastro-intestinal tract, in particular by means of oral administration or other administration into the gastrointestinal tract, it will usually not be necessary to use polypeptides of the invention that have increased half-life in serum (i.e. that have been pegylated or that contain a Nanobody directed against a serum protein). Thus, for such indications, polypeptides of the invention can be used that only contain Nanobodies of the invention. In particular, it has been found that for oral administration for the prevention and treatment of diseases or disorders of the gastro-intestinal tract associated with and/or mediated by TNF-alpha (such as IBD and the other diseases and disorders of the gastro-intestinal tract mentioned above), the use of a monovalent Nanobody of the invention or of a polypeptide of the invention that essentially consists of a monovalent Nanobody of the invention may be preferred. For other indications, such as the treatment of rheumatoid arthritis (RA), the use of a bivalent Nanobody of the invention may be preferred. When such a Nanobody has to reach its intended site of action via the blood stream, the use of a polypeptide of the invention that has increased half-life in serum may be preferred.

A subject according to the invention can be any mammal susceptible to treatment by therapeutic polypeptides.

Oral delivery of anti-TNF-alpha polypeptides of the invention results in the provision of such molecules in an active form in the colon at local sites that are affected by the disorder. These sites may be highly inflamed and contain TNF-alpha-producing cells. The anti-TNF-alpha polypeptides of the invention which bind to TNF-alpha can neutralise the TNF-alpha locally, avoiding distribution throughout the whole body and thus limiting negative side-effects. Genetically modified microorganisms such as Micrococcus lactis are able to secrete antibody or functional portions thereof. Such modified microorganisms can be used as vehicles for local production and delivery of antibodies or functional portions thereof

in the intestine. By using a strain which produces an anti-TNF-alpha polypeptide, inflammatory bowel syndrome could be treated.

Another aspect of the invention involves delivering anti-TNF polypeptides by using surface expression on or secretion from non-invasive bacteria, such as Gram-positive host organisms like Lactococcus spec. using a vector such as described in WO00/23471.

One embodiment of the present invention is an anti-TNF-alpha polypeptide as disclosed herein for use in treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention which is able pass through the gastric environment without the substance being inactivated.

Examples of disorders are any that cause inflammation, including, but not limited to rheumatoid arthritis, Crohn's disease, ulcerative colitis, inflammatory bowel syndrome, and multiple sclerosis. As known by persons skilled in the art, once in possession of said polypeptide construct, formulation technology may be applied to release a maximum amount of polypeptide in the right location (in the stomach, in the colon, etc.). This method of delivery is important for treating, prevent and/or alleviate the symptoms of disorders whose targets are located in the gut system.

An aspect of the invention is a method for treating, preventing and/or alleviating the symptoms of a disorder susceptible to modulation by a Nanobody or polypeptide of the invention which is able pass through the gastric environment without being inactivated, by orally administering to a subject an anti-TNF-alpha polypeptide as disclosed herein.

Another embodiment of the present invention is a use of an anti-TNF-alpha polypeptide as disclosed herein for the preparation of a medicament for treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention which is able pass through the gastric environment without being inactivated.

An aspect of the invention is a method for delivering a Nanobody or polypeptide of the invention to the gut system without said substance being inactivated, by orally administering to a subject an anti-TNF-alpha polypeptide as disclosed herein.

An aspect of the invention is a method for delivering a Nanobody or polypeptide of the invention to the bloodstream of a subject without the substance being inactivated, by orally administering to a subject an anti-TNF-alpha polypeptide as disclosed herein.

Another embodiment of the present invention is an anti-TNF-alpha polypeptide as disclosed herein for use in treating, preventing and/or alleviating the symptoms or disorders

susceptible to modulation by a Nanobody or polypeptide of the invention delivered to the vaginal and/or rectal tract.

Examples of disorders are any that cause inflammation, including, but not limited to rheumatoid arthritis, Crohn's disease, ulcerative colitis, inflammatory bowel syndrome, and multiple sclerosis. In a non-limiting example, a formulation according to the invention comprises an anti-TNF-alpha polypeptide as disclosed herein, in the form of a gel, cream, suppository, film, or in the form of a sponge or as a vaginal ring that slowly releases the active ingredient over time (such formulations are described in EP 707473, EP 684814, US 5629001).

An aspect of the invention is a method for treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention delivered to the vaginal and/or rectal tract, by vaginally and/or rectally administering to a subject an anti-TNF-alpha polypeptide as disclosed herein.

Another embodiment of the present invention is a use of an anti-TNF-alpha polypeptide as disclosed herein for the preparation of a medicament for treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention delivered to the vaginal and/or rectal tract.

An aspect of the invention is a method for delivering a Nanobody or polypeptide of the invention to the vaginal and/or rectal tract without being said substance being inactivated, by administering to the vaginal and/or rectal tract of a subject an anti-TNF-alpha polypeptide as disclosed herein.

An aspect of the invention is a method for delivering a Nanobody or polypeptide of the invention to the bloodstream of a subject without said substance being inactivated, by administering to the vaginal and/or rectal tract of a subject an anti-TNF-alpha polypeptide as disclosed herein.

Another embodiment of the present invention is an anti-TNF-alpha polypeptide as disclosed herein, for use in treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention delivered to the nose, upper respiratory tract and/or lung.

Examples of disorders are any that cause inflammation, including, but not limited to rheumatoid arthritis, Crohn's disease, ulcerative colitis, inflammatory bowel syndrome, and multiple sclerosis. In a non-limiting example, a formulation according to the invention, comprises an anti-TNF-alpha polypeptide as disclosed herein in the form of a nasal spray

(e.g. an aerosol) or inhaler. Since the polypeptide construct is small, it can reach its target much more effectively than therapeutic IgG molecules.

An aspect of the invention is a method for treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention delivered to the upper respiratory tract and lung, by administering to a subject an anti-TNF-alpha polypeptide as disclosed herein, by inhalation through the mouth or nose.

Another embodiment of the present invention is a use of an anti-TNF-alpha polypeptide as disclosed herein for the preparation of a medicament for treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention delivered to the nose, upper respiratory tract and/or lung, without said polypeptide being inactivated.

An aspect of the invention is a method for delivering a Nanobody or polypeptide of the invention to the nose, upper respiratory tract and lung without inactivation, by administering to the nose, upper respiratory tract and/or lung of a subject an anti-TNF-alpha polypeptide as disclosed herein.

An aspect of the invention is a method for delivering a Nanobody or polypeptide of the invention to the bloodstream of a subject without inactivation by administering to the nose, upper respiratory tract and/or lung of a subject an anti-TNF-alpha polypeptide as disclosed herein.

One embodiment of the present invention is an anti-TNF-alpha polypeptide as disclosed herein for use in treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention delivered to the intestinal mucosa, wherein said disorder increases the permeability of the intestinal mucosa. Because of their small size, an anti-TNF-alpha polypeptide as disclosed herein can pass through the intestinal mucosa and reach the bloodstream more efficiently in subjects suffering from disorders which cause an increase in the permeability of the intestinal mucosa, for example Crohn's disease.

An aspect of the invention is a method for treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention delivered to the intestinal mucosa, wherein said disorder increases the permeability of the intestinal mucosa, by orally administering to a subject an anti-TNF-alpha polypeptide as disclosed herein.

This process can be even further enhanced by an additional aspect of the present invention - the use of active transport carriers. In this aspect of the invention, V_{HH} is fused to a carrier that enhances the transfer through the intestinal wall into the bloodstream. In a non-limiting example, this "carrier" is a second V_{HH} which is fused to the therapeutic V_{HH}. Such fusion constructs are made using methods known in the art. The "carrier" V_{HH} binds specifically to a receptor on the intestinal wall which induces an active transfer through the wall.

Another embodiment of the present invention is a use of an anti-TNF-alpha polypeptide as disclosed herein for the preparation of a medicament for treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention delivered to the intestinal mucosa, wherein said disorder increases the permeability of the intestinal mucosa.

An aspect of the invention is a method for delivering a Nanobody or polypeptide of the invention to the intestinal mucosa without being inactivated, by administering orally to a subject an anti-TNF-alpha polypeptide of the invention.

An aspect of the invention is a method for delivering a Nanobody or polypeptide of the invention to the bloodstream of a subject without being inactivated, by administering orally to a subject an anti-TNF-alpha polypeptide of the invention.

This process can be even further enhanced by an additional aspect of the present invention - the use of active transport carriers. In this aspect of the invention, an anti-TNF-alpha polypeptide as described herein is fused to a carrier that enhances the transfer through the intestinal wall into the bloodstream. In a non-limiting example, this "carrier" is a V_{HH} which is fused to said polypeptide. Such fusion constructs made using methods known in the art. The "carrier" V_{HH} binds specifically to a receptor on the intestinal wall which induces an active transfer through the wall.

One embodiment of the present invention is an anti-TNF-alpha polypeptide as disclosed herein for use in treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention which is able pass through the tissues beneath the tongue effectively.

Examples of disorders are any that cause inflammation, including, but not limited to rheumatoid arthritis, Crohn's disease, ulcerative colitis, inflammatory bowel syndrome, and multiple sclerosis. A formulation of said polypeptide construct as disclosed herein, for

example, a tablet, spray, drop is placed under the tongue and adsorbed through the mucus membranes into the capillary network under the tongue.

An aspect of the invention is a method for treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention which is able pass through the tissues beneath the tongue effectively, by sublingually administering to a subject an anti-TNF-alpha polypeptide as disclosed herein.

Another embodiment of the present invention is a use of an anti-TNF-alpha polypeptide as disclosed herein for the preparation of a medicament for treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention which is able to pass through the tissues beneath the tongue.

An aspect of the invention is a method for delivering a Nanobody or polypeptide of the invention to the tissues beneath the tongue without being inactivated, by administering sublingually to a subject an anti-TNF-alpha polypeptide as disclosed herein.

An aspect of the invention is a method for delivering a Nanobody or polypeptide of the invention to the bloodstream of a subject without being inactivated, by administering orally to a subject an anti-TNF-alpha polypeptide as disclosed herein.

One embodiment of the present invention is an anti-TNF-alpha polypeptide as disclosed herein for use in treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention which is able pass through the skin effectively.

Examples of disorders are any that cause inflammation, including, but not limited to rheumatoid arthritis, Crohn's disease, ulcerative colitis, inflammatory bowel syndrome, and multiple sclerosis. A formulation of said polypeptide construct, for example, a cream, film, spray, drop, patch, is placed on the skin and passes through.

An aspect of the invention is a method for treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention which is able pass through the skin effectively, by topically administering to a subject an anti-TNF-alpha polypeptide as disclosed herein.

Another embodiment of the present invention is a use of an anti-TNF-alpha polypeptide as disclosed herein for the preparation of a medicament for treating, preventing and/or alleviating the symptoms of disorders susceptible to modulation by a Nanobody or polypeptide of the invention which is able pass through the skin effectively.

An aspect of the invention is a method for delivering a Nanobody or polypeptide of the invention to the skin without being inactivated, by administering topically to a subject an anti-TNF-alpha polypeptide as disclosed herein.

An aspect of the invention is a method for delivering a Nanobody or polypeptide of the invention to the bloodstream of a subject, by administering topically to a subject an anti-TNF-alpha polypeptide as disclosed herein.

In another embodiment of the present invention, an anti-TNF-alpha polypeptide further comprises a carrier nanobody (e.g. V_{HH}) which acts as an active transport carrier for transport said anti-TNF-alpha polypeptide, from the lung lumen to the blood.

An anti-TNF-alpha polypeptide further comprising a carrier binds specifically to a receptor present on the mucosal surface (bronchial epithelial cells) resulting in the active transport of the polypeptide from the lung lumen to the blood. The carrier nanobody may be fused to the polypeptide construct. Such fusion constructs may be made using methods known in the art and are describe herein. The "carrier" nanobody binds specifically to a receptor on the mucosal surface which induces an active transfer through the surface.

Another aspect of the present invention is a method to determine which nanobodies (e.g. V_{HH}s) are actively transported into the bloodstream upon nasal administration. Similarly, a naïve or immune V_{HH} phage library can be administered nasally, and after different time points after administration, blood or organs can be isolated to rescue phages that have been actively transported to the bloodstream. A non-limiting example of a receptor for active transport from the lung lumen to the bloodstream is the Fc receptor N (FcRn). One aspect of the invention includes the V_{HH} molecules identified by the method. Such V_{HH} can then be used as a carrier V_{HH} for the delivery of a therapeutic V_{HH} to the corresponding target in the bloodstream upon nasal administration.

In one aspect of the invention, one can use an anti-TNF-alpha polypeptide as disclosed herein, in order to screen for agents that modulate the binding of the polypeptide to TNF-alpha. When identified in an assay that measures binding or said polypeptide displacement alone, agents will have to be subjected to functional testing to determine whether they would modulate the action of the antigen in vivo.

In an example of a displacement experiment, phage or cells expressing TNF-alpha or a fragment thereof are incubated in binding buffer with polypeptide of the invention which has been labeled, in the presence or absence of increasing concentrations of a candidate modulator. To validate and calibrate the assay, control competition reactions using

increasing concentrations of said polypeptide and which is unlabeled, can be performed. After incubation, cells are washed extensively, and bound, labeled polypeptide is measured as appropriate for the given label (e.g., scintillation counting, fluorescence, etc.). A decrease of at least 10% in the amount of labeled polypeptide bound in the presence of candidate modulator indicates displacement of binding by the candidate modulator. Candidate modulators are considered to bind specifically in this or other assays described herein if they displace 50% of labeled polypeptide (sub-saturating polypeptide dose) at a concentration of 1 μM or less.

Alternatively, binding or displacement of binding can be monitored by surface plasmon resonance (SPR). Surface plasmon resonance assays can be used as a quantitative method to measure binding between two molecules by the change in mass near an immobilized sensor caused by the binding or loss of binding of the polypeptide of the invention from the aqueous phase to TNF-alpha immobilized in a membrane on the sensor. This change in mass is measured as resonance units versus time after injection or removal of the said polypeptide or candidate modulator and is measured using a Biacore Biosensor (Biacore AB). TNF-alpha can be for example immobilized on a sensor chip (for example, research grade CM5 chip; Biacore AB) in a thin film lipid membrane according to methods described by Salamon et al. (Salamon et al., 1996, Biophys J. 71: 283-294; Salamon et al., 2001, Biophys. J. 80: 1557-1567; Salamon et al., 1999, Trends Biochem. Sci. 24: 213-219, each of which is incorporated herein by reference.). Sarrio et al. demonstrated that SPR can be used to detect ligand binding to the GPCR A(1) adenosine receptor immobilized in a lipid layer on the chip (Sarrio et al., 2000, Mol. Cell. Biol. 20: 5164-5174, incorporated herein by reference). Conditions for the binding of a polypeptide of the invention to TNF-alpha in an SPR assay can be fine-tuned by one of skill in the art using the conditions reported by Sarrio et al. as a starting point.

SPR can assay for modulators of binding in at least two ways. First, a polypeptide of the invention can be pre-bound to immobilized TNF-alpha followed by injection of candidate modulator at a concentration ranging from 0.1 nM to 1 μM. Displacement of the bound polypeptide can be quantitated, permitting detection of modulator binding. Alternatively, the membrane-bound TNF-alpha can be pre-incubated with a candidate modulator and challenged with the polypeptide of the invention. A difference in binding affinity between said polypeptide and TNF-alpha pre-incubated with the modulator, compared with that between said polypeptide and TNF-alpha in absence of the modulator

will demonstrate binding or displacement of said polypeptide in the presence of modulator. In either assay, a decrease of 10% or more in the amount of said polypeptide bound in the presence of candidate modulator, relative to the amount of said polypeptide bound in the absence of candidate modulator indicates that the candidate modulator inhibits the interaction of TNF-alpha and said polypeptide.

Another method of detecting inhibition of binding of, for example, a polypeptide polypeptide of the invention, to TNF-alpha uses fluorescence resonance energy transfer (FRET). FRET is a quantum mechanical phenomenon that occurs between a fluorescence donor (D) and a fluorescence acceptor (A) in close proximity to each other (usually < 100 Å of separation) if the emission spectrum of D overlaps with the excitation spectrum of A. The molecules to be tested, e.g. a polypeptide polypeptide of the invention and a TNF-alpha are labelled with a complementary pair of donor and acceptor fluorophores. While bound closely together by the TNF-alpha: polypeptide interaction, the fluorescence emitted upon excitation of the donor fluorophore will have a different wavelength from that emitted in response to that excitation wavelength when the said polypeptide and TNF-alpha are not bound, providing for quantitation of bound versus unbound molecules by measurement of emission intensity at each wavelength. Donor fluorophores with which to label the TNF-alpha are well known in the art. Of particular interest are variants of the A. Victoria GFP known as Cyan FP (CFP, Donor (D)) and Yellow FP (YFP, Acceptor (A)). As an example, the YFP variant can be made as a fusion protein with TNF-alpha. Vectors for the expression of GFP variants as fusions (Clontech) as well as fluorophore-labeled reagents (Molecular Probes) are known in the art. The addition of a candidate modulator to the mixture of fluorescently-labelled polypeptide and YFP-TNF-alpha will result in an inhibition of energy transfer evidenced by, for example, a decrease in YFP fluorescence relative to a sample without the candidate modulator. In an assay using FRET for the detection of TNF-alpha: polypeptide interaction, a 10% or greater decrease in the intensity of fluorescent emission at the acceptor wavelength in samples containing a candidate modulator, relative to samples without the candidate modulator, indicates that the candidate modulator inhibits the TNF-alpha:polypeptide interaction.

A sample as used herein may be any biological sample containing TNF-alpha such as clinical (e.g. cell fractions, whole blood, plasma, serum, tissue, cells, etc.), derived from clinical, agricultural, forensic, research, or other possible samples. The clinical samples may

be from human or animal origin. The sample analysed can be both solid or liquid in nature. It is evident when solid materials are used, these are first dissolved in a suitable solution.

A variation on FRET uses fluorescence quenching to monitor molecular interactions. One molecule in the interacting pair can be labelled with a fluorophore, and the other with a molecule that quenches the fluorescence of the fluorophore when brought into close apposition with it. A change in fluorescence upon excitation is indicative of a change in the association of the molecules tagged with the fluorophore:quencher pair. Generally, an increase in fluorescence of the labelled TNF-alpha is indicative that anti-TNF-alpha polypeptide bearing the quencher has been displaced. For quenching assays, a 10% or greater increase in the intensity of fluorescent emission in samples containing a candidate modulator, relative to samples without the candidate modulator, indicates that the candidate modulator inhibits TNF-alpha: anti-TNF-alpha polypeptide interaction.

In addition to the surface plasmon resonance and FRET methods, fluorescence polarization measurement is useful to quantitate binding. The fluorescence polarization value for a fluorescently-tagged molecule depends on the rotational correlation time or tumbling rate. Complexes, such as those formed by TNF-alpha associating with a fluorescently labelled anti-TNF-alpha polypeptide, have higher polarization values than uncomplexed, labelled polypeptide. The inclusion of a candidate inhibitor of the TNF-alpha:anti-TNF-alpha polypeptide interaction results in a decrease in fluorescence polarization, relative to a mixture without the candidate inhibitor, if the candidate inhibitor disrupts or inhibits the interaction of TNF-alpha with said polypeptide. Fluorescence polarization is well suited for the identification of small molecules that disrupt the formation of TNF-alpha:anti-TNF-alpha polypeptide complexes. A decrease of 10% or more in fluorescence polarization in samples containing a candidate modulator, relative to fluorescence polarization in a sample lacking the candidate modulator, indicates that the candidate modulator inhibits the TNF-alpha : anti-TNF-alpha polypeptide interaction.

Another alternative for monitoring TNF-alpha : anti-TNF-alpha polypeptide interactions uses a biosensor assay. ICS biosensors have been described in the art (Australian Membrane Biotechnology Research Institute; Cornell B, Braach-Maksvytis V, King L, Osman P, Raguse B, Wieczorek L, and Pace R. "A biosensor that uses ion-channel switches" Nature 1997, 387, 580). In this technology, the association of TNF-alpha and a anti-TNF-alpha polypeptide is coupled to the closing of gramacidin-facilitated ion channels in suspended membrane bilayers and thus to a measurable change in the admittance (similar to

impedence) of the biosensor. This approach is linear over six orders of magnitude of admittance change and is ideally suited for large scale, high throughput screening of small molecule combinatorial libraries. A 10% or greater change (increase or decrease) in admittance in a sample containing a candidate modulator, relative to the admittance of a sample lacking the candidate modulator, indicates that the candidate modulator inhibits the interaction of TNF-alpha and said polypeptide. It is important to note that in assays testing the interaction of TNF-alpha with an anti-TNF-alpha polypeptide, it is possible that a modulator of the interaction need not necessarily interact directly with the domain(s) of the proteins that physically interact with said polypeptide. It is also possible that a modulator will interact at a location removed from the site of interaction and cause, for example, a conformational change in the TNF-alpha. Modulators (inhibitors or agonists) that act in this manner are nonetheless of interest as agents to modulate the binding of TNF-alpha to its receptor.

Any of the binding assays described can be used to determine the presence of an agent in a sample, e.g., a tissue sample, that binds to TNF-alpha , or that affects the binding of, for example, a polypeptide polypeptide of the invention to the TNF-alpha. To do so a TNF-alpha is reacted with said polypeptide in the presence or absence of the sample, and polypeptide binding is measured as appropriate for the binding assay being used. A decrease of 10% or more in the binding of said polypeptide indicates that the sample contains an agent that modulates the binding of said polypeptide to the TNF-alpha. Of course, the above-generalized method might easily be applied to screening for candidate modulators which alter the binding between any anti-TNF-alpha polypeptide of the invention, an homologous sequence thereof, a functional portion thereof or a functional portion of an homologous sequence thereof, and TNF-alpha or a fragment thereof.

One embodiment of the present invention is an unknown agent identified by the method disclosed herein.

One embodiment of the present invention is an unknown agent identified by the method disclosed herein for use in treating, preventing and/or alleviating the symptoms of disorders relating to inflammatory processes.

Another embodiment of the present invention is a use of an unknown agent identified by the method disclosed herein for use in treating, preventing and/or alleviating the symptoms of disorders relating to inflammatory processes.

Examples of disorders include rheumatoid arthritis, Crohn's disease, ulcerative colitis, inflammatory bowel syndrome and multiple sclerosis.

A cell that is useful according to the invention is preferably selected from the group consisting of bacterial cells such as, for example, E. coli, yeast cells such as, for example, S. cerevisiae, P. pastoris, insect cells or mammal cells.

A cell that is useful according to the invention can be any cell into which a nucleic acid sequence encoding a polypeptide comprising an anti-TNF-alpha of the invention, an homologous sequence thereof, a functional portion thereof or a functional portion of an homologous sequence thereof according to the invention can be introduced such that the polypeptide is expressed at natural levels or above natural levels, as defined herein. Preferably a polypeptide of the invention that is expressed in a cell exhibits normal or near normal pharmacology, as defined herein.

According to a preferred embodiment of the present invention, a cell is selected from the group consisting of COS7-cells, a CHO cell, a LM (TK-) cell, a NIH-3T3 cell, HEK-293 cell, K-562 cell or a 1321N1 astrocytoma cell but also other transfectable cell lines.

In general, "therapeutically effective amount", "therapeutically effective dose" and "effective amount" means the amount needed to achieve the desired result or results (modulating TNF-alpha binding; treating or preventing inflammation). One of ordinary skill in the art will recognize that the potency and, therefore, an "effective amount" can vary for the various compounds that modulate TNF-alpha binding used in the invention. One skilled in the art can readily assess the potency of the compound.

As used herein, the term "compound" refers to an anti-TNF-alpha polypeptide of the present invention, a composition, or a nucleic acid capable of encoding said polypeptide or an agent identified according to the screening method described herein or said polypeptide comprising one or more derivatised amino acids.

By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to an individual along with the compound without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained.

Anti-TNF-alpha polypeptides as disclosed herein is useful for treating or preventing conditions in a subject and comprises administering a pharmaceutically effective amount of a compound or composition.

Anti-TNF polypeptides of the present invention are useful for treating or preventing conditions relating to rheumatoid arthritis, Crohn's disease, ulcerative colitis, inflammatory bowel syndrome and multiple sclerosis in a subject and comprises administering a pharmaceutically effective amount of a compound or composition that binds TNF-alpha.

Anti-TNF-alpha polypeptides as disclosed here in are useful for treating or preventing conditions in a subject and comprises administering a pharmaceutically effective amount of a compound combination with another, such as, for example, aspirin.

The anti-TNF-alpha polypeptides as disclosed here in are useful for treating or preventing conditions relating to rheumatoid arthritis, Crohn's disease, ulcerative colitis, inflammatory bowel syndrome and multiple sclerosis in a subject and comprises administering a pharmaceutically effective amount of a compound combination with another, such as, for example, aspirin.

The present invention is not limited to the administration of formulations comprising a single compound of the invention. It is within the scope of the invention to provide combination treatments wherein a formulation is administered to a patient in need thereof that comprises more than one compound of the invention.

Conditions mediated by TNF-alpha include, but are not limited rheumatoid arthritis, Crohn's disease, ulcerative colitis, inflammatory bowel syndrome and multiple sclerosis.

A compound useful in the present invention can be formulated as pharmaceutical compositions and administered to a mammalian host, such as a human patient or a domestic animal in a variety of forms adapted to the chosen route of administration, i.e., orally or parenterally, by intranassally by inhalation, intravenous, intramuscular, topical or subcutaneous routes.

A compound of the present invention can also be administered using gene therapy methods of delivery. See, e.g., U.S. Patent No. 5,399,346, which is incorporated by reference in its entirety. Using a gene therapy method of delivery, primary cells transfected with the gene for the compound of the present invention can additionally be transfected with tissue specific promoters to target specific organs, tissue, grafts, tumors, or cells.

Thus, the present compound may be systemically administered, e.g., orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the active compound may be combined with one or more

excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of active compound in such therapeutically useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and devices.

The active compound may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the active compound or its salts can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form must be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water,

ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

For topical administration, the present compound may be applied in pure form, i.e., when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Useful liquid carriers include water, hydroxyalkyls or glycols or water-alcohol/glycol blends, in which the present compound can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

Examples of useful dermatological compositions which can be used to deliver the compound to the skin are known to the art; for example, see Jacquet et al. (U.S. Pat. No. 4,608,392), Geria (U.S. Pat. No. 4,992,478), Smith et al. (U.S. Pat. No. 4,559,157) and Wortzman (U.S. Pat. No. 4,820,508).

Useful dosages of the compound can be determined by comparing their in vitro activity, and in vivo activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949.

Generally, the concentration of the compound(s) in a liquid composition, such as a lotion, will be from about 0.1-25 wt-%, preferably from about 0.5-10 wt-%. The concentration in a semi-solid or solid composition such as a gel or a powder will be about 0.1-5 wt-%, preferably about 0.5-2.5 wt-%.

The amount of the compound, or an active salt or derivative thereof, required for use in treatment will vary not only with the particular salt selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician. Also the dosage of the compound varies depending on the target cell, tumor, tissue, graft, or organ.

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations; such as multiple inhalations from an insufflator or by application of a plurality of drops into the eye.

An administration regimen could include long-term, daily treatment. By "long-term" is meant at least two weeks and preferably, several weeks, months, or years of duration. Necessary modifications in this dosage range may be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein. See Remington's Pharmaceutical Sciences (Martin, E.W., ed. 4), Mack Publishing Co., Easton, PA. The dosage can also be adjusted by the individual physician in the event of any complication.

The invention provides for an agent that is a modulator of TNF-alpha / TNF-alpha-receptor interactions.

The candidate agent may be a synthetic agent, or a mixture of agents, or may be a natural product (e.g. a plant extract or culture supernatant). A candidate agent according to

the invention includes a small molecule that can be synthesized, a natural extract, peptides, proteins, carbohydrates, lipids etc.

Candidate modulator agents from large libraries of synthetic or natural agents can be screened. Numerous means are currently used for random and directed synthesis of saccharide, peptide, and nucleic acid based agents. Synthetic agent libraries are commercially available from a number of companies including Maybridge Chemical Co. (Trevillet, Cornwall, UK), Comgenex (Princeton, NJ), Brandon Associates (Merrimack, NH), and Microsource (New Milford, CT). A rare chemical library is available from Aldrich (Milwaukee, WI). Combinatorial libraries are available and can be prepared. Alternatively, libraries of natural agents in the form of bacterial, fungal, plant and animal extracts are available from e.g., Pan Laboratories (Bothell, WA) or MycoSearch (NC), or are readily producible by methods well known in the art. Additionally, natural and synthetically produced libraries and agents are readily modified through conventional chemical, physical, and biochemical means.

Useful agents may be found within numerous chemical classes. Useful agents may be organic agents, or small organic agents. Small organic agents have a molecular weight of more than 50 yet less than about 2,500 daltons, preferably less than about 750, more preferably less than about 350 daltons. Exemplary classes include heterocycles, peptides, saccharides, steroids, and the like. The agents may be modified to enhance efficacy, stability, pharmaceutical compatibility, and the like. Structural identification of an agent may be used to identify, generate, or screen additional agents. For example, where peptide agents are identified, they may be modified in a variety of ways to enhance their stability, such as using an unnatural amino acid, such as a D-amino acid, particularly D-alanine, by functionalizing the amino or carboxylic terminus, e.g. for the amino group, acylation or alkylation, and for the carboxyl group, esterification or amidification, or the like.

For primary screening, a useful concentration of a candidate agent according to the invention is from about 10 mM to about 100 μM or more (i.e. 1 mM, 10 mM, 100 mM, 1 M etc.). The primary screening concentration will be used as an upper limit, along with nine additional concentrations, wherein the additional concentrations are determined by reducing the primary screening concentration at half-log intervals (e.g. for 9 more concentrations) for secondary screens or for generating concentration curves.

A high throughput screening kit according to the invention comprises all the necessary means and media for performing the detection of an agent that modulates TNF-

alpha/TNF-alpha receptor interactions by interacting with TNF-alpha in the presence of a polypeptide, preferably at a concentration in the range of 1μM to 1 mM.

The kit comprises the following. Recombinant cells of the invention, comprising and expressing the nucleotide sequence encoding TNF-alpha , which are grown according to the kit on a solid support, such as a microtiter plate, more preferably a 96 well microtiter plate, according to methods well known to the person skilled in the art especially as described in WO 00/02045. Alternatively TNF-alpha is supplied in a purified form to be immobilized on, for example, a 96 well microtiter plate by the person skilled in the art. Alternatively TNF-alpha is supplied in the kit pre-immobilized on, for example, a 96 well microtiter plate. The TNF-alpha may be whole TNF-alpha or a fragment thereof.

Modulator agents according to the invention, at concentrations from about 1 μM to 1 mM or more, are added to defined wells in the presence of an appropriate concentration of anti-TNF-alpha polypeptide, an homologous sequence thereof, a functional portion thereof or a functional portion of an homologous sequence thereof, said concentration of said polypeptide preferably in the range of 1 μM to 1 mM. Kits may contain one or more anti-TNF-alpha polypeptides of the invention.

Binding assays are performed as according to the methods already disclosed herein and the results are compared to the baseline level of, for example TNF-alpha binding to an anti-TNF-alpha polypeptide, an homologous sequence thereof, a functional portion thereof or a functional portion of an homologous sequence thereof , but in the absence of added modulator agent. Wells showing at least 2 fold, preferably 5 fold, more preferably 10 fold and most preferably a 100 fold or more increase or decrease in TNF-alpha-polypeptide binding (for example) as compared to the level of activity in the absence of modulator, are selected for further analysis.

The invention provides for other kits useful for screening for modulators of TNF-alpha/TNF-alpha receptor binding, as well as kits useful for diagnosis of disorders characterised by dysfunction of TNF-alpha. The invention also provides for kits useful for screening for modulators of disorders as well as kits for their diagnosis, said disorders characterised by one or more process involving TNF-alpha. Kits useful according to the invention can include an isolated TNF-alpha . Alternatively, or in addition, a kit can comprise cells transformed to express TNF-alpha. In a further embodiment, a kit according to the invention can comprise a polynucleotide encoding TNF-alpha. In a still further embodiment, a kit according to the invention may comprise the specific primers useful for amplification of

TNF-alpha. Kits useful according to the invention can comprise an isolated TNF-alpha polypeptide, a homologue thereof, or a functional portion thereof. A kit according to the invention can comprise cells transformed to express said polypeptide. Kits may contain more than one polypeptide. In a further embodiment, a kit according to the invention can comprise a polynucleotide encoding TNF-alpha . In a still further embodiment, a kit according to the invention may comprise the specific primers useful for amplification of a macromolecule such as, for example, TNF-alpha. All kits according to the invention will comprise the stated items or combinations of items and packaging materials therefore. Kits will also include instructions for use.

Furthermore, it will also be clear to the skilled person that it may be possible to "graft" one or more of the CDR's mentioned above for the Nanobodies of the invention onto other "scaffolds", including but not limited to human scaffolds or non-immunoglobulin scaffolds. Suitable scaffolds and techniques for such CDR grafting will be clear to the skilled person and are well known in the art, see for example US-A-7,180,370, WO 01/27160, EP 0 605 522, EP 0 460 167, US-A-7,054,297, Nicaise et al., Protein Science (2004), 13:1882-1891; Ewert et al., Methods, 2004 Oct; 34(2):184-199; Kettleborough et al., Protein Eng. 1991 Oct; 4(7): 773-783; O'Brien and Jones, Methods Mol. Biol. 2003: 207: 81-100; and Skerra, J. Mol. Recognit. 2000: 13: 167-187, and Saerens et al., J. Mol. Biol. 2005 Sep 23;352(3):597-607, and the further references cited therein. For example, techniques known per se for grafting mouse or rat CDR's onto human frameworks and scaffolds can be used in an analogous manner to provide chimeric proteins comprising one or more of the CDR's of the Nanobodies of the invention and one or human framework regions or sequences.

Thus, in another embodiment, the invention comprises a chimeric polypeptide comprising at least one CDR sequence chosen from the group consisting of CDR1 sequences, CDR2 sequences and CDR3 sequences mentioned herein for the Nanobodies of the invention. Preferably, such a chimeric polypeptide comprises at least one CDR sequence chosen from the group consisting of the CDR3 sequences mentioned herein for the Nanobodies of the invention, and optionally also at least one CDR sequence chosen from the group consisting of the CDR1 sequences and CDR2 sequences mentioned herein for the Nanobodies of the invention. For example, such a chimeric polypeptide may comprise one CDR sequence chosen from the group consisting of the CDR3 sequences mentioned herein for the Nanobodies of the invention, one CDR sequence chosen from the group consisting of the CDR1 sequences mentioned herein for the Nanobodies of the invention and one CDR

sequence chosen from the group consisting of the CDR1 sequences and CDR2 sequences mentioned herein for the Nanobodies of the invention. The combinations of CDR's that are mentioned herein as being preferred for the Nanobodies of the invention will usually also be preferred for these chimeric polypeptides.

In said chimeric polypeptides, the CDR's may be linked to further amino acid sequences sequences and/or may be linked to each other via amino acid sequences, in which said amino acid sequences are preferably framework sequences or are amino acid sequences that act as framework sequences, or together form a scaffold for presenting the CDR's. Reference is again made to the prior art mentioned in the last paragraph. According to one preferred embodiment, the amino acid sequences are human framework sequences, for example V_H3 framework sequences. However, non-human, synthetic, semi-synthetic or non-immunoglobulin framework sequences may also be used. Preferably, the framework sequences used are such that (1) the chimeric polypeptide is capable of binding xxxx, i.e. with an affinity that is at least 1%, preferably at least 5%, more preferably at least 10%, such as at least 25% and up to 50% or 90% or more of the affinity of the corresponding Nanobody of the invention; (2) the chimeric polypeptide is suitable for pharmaceutical use; and (3) the chimeric polypeptide is preferably essentially non-immunogenic under the intended conditions for pharmaceutical use (i.e. indication, mode of administration, dosis and treatment regimen) thereof (which may be essentially analogous to the conditions described herein for the use of the Nanobodies of the invention).

According to one non-limiting embodiment, the chimeric polypeptide comprises at least two CDR sequences (as mentioned above) linked via at least one framework sequence, in which preferably at least one of the two CDR sequences is a CDR3 sequence, with the other CDR sequence being a CDR1 or CDR2 sequence. According to a preferred, but non-limiting embodiment, the chimeric polypeptide comprises at least two CDR sequences (as mentioned above) linked at least two framework sequences, in which preferably at least one of the three CDR sequences is a CDR3 sequence, with the other two CDR sequences being CDR1 or CDR2 sequences, and preferably being one CDR1 sequence and one CDR2 sequence. According to one specifically preferred, but non-limiting embodiment, the chimeric polypeptides have the structure FR1' - CDR1 - FR2' - CDR2 - FR3' - CDR3 - FR4', in which CDR1, CDR2 and CDR3 are as defined herein for the CDR's of the Nanobodies of the invention, and FR1', FR2', FR3' and FR4' are framework sequences. FR1', FR2', FR3' and FR4' may in particular be Framework 1, Framework 2, Framework 3 and Framework 4

sequences, respectively, of a human antibody (such as V_H3 sequences) and/or parts or fragments of such Framework sequences. It is also possible to use parts or fragments of a chimeric polypeptide with the structure FR1' - CDR1 - FR2' - CDR2 - FR3' - CDR3 - FR4. Preferably, such parts or fragments are such that they meet the criteria set out in the preceding paragraph.

The invention also relates to proteins and polypeptides comprising and/or essentially consisting of such chimeric polypeptides, to nucleic acids encoding such proteins or polypeptides; to methods for preparing such proteins and polypeptides; to host cells expressing or capable of expressing such proteins or polypeptides; to compositions, and in particular to pharmaceutical compositions, that comprise such proteins or polypeptides, nucleic acids or host cells; and to uses of such proteins or polypeptides, such nucleic acids, such host cells and/or such compositions, in particular for prophylactic, therapeutic or diagnostic purposes, such as the prophylactic, therapeutic or diagnostic purposes mentioned herein. For example, such proteins, polypeptides, nucleic acids, methods, host cells, compositions and uses may be analogous to the proteins, polypeptides, nucleic acids, methods, host cells, compositions and use described herein for the Nanobodies of the invention.

It should also be noted that, when the Nanobodies of the inventions contain one or more other CDR sequences than the preferred CDR sequences mentioned above, these CDR sequences can be any suitable (i.e. suitable for the purposes described herein) CDR sequences and/or these CDR sequences can be obtained in any manner known per se, for example from Nanobodies (preferred), V_H domains from conventional antibodies (and in particular from human antibodies), heavy chain antibodies, conventional 4-chain antibodies (such as conventional human 4-chain antibodies) or other immunoglobulin sequences directed against TNF. Such immunoglobulin sequences directed against xxxx can be generated in any manner known per se, as will be clear to the skilled person, i.e. by immunization with TNF or by screening a suitable library of immunoglobulin sequences with TNF, or any suitable combination thereof. Optionally, this may be followed by techniques such as random or site-directed mutagenesis and/or other techniques for affinity maturation known per se. Suitable techniques for generating such immunoglobulin sequences will be clear to the skilled person, and for example include the screening techniques reviewed by Hoogenboom, Nature Biotechnology, 23, 9, 1105-1116 (2005) . Other techniques for generating immunoglobulins against a specified target include for example the Nanoclone technology (as for example

described in the non-prepublished US provisional patent application 60/648,922), so-called SLAM technology (as for example described in the European patent application 0 542 810), the use of transgenic mice expressing human immunoglobulins or the well-known hybridoma techniques (see for example Larrick et al, Biotechnology, Vol.7, 1989, p. 934). All these techniques can be used to generate immunoglobulins against TNF, and the CDR's of such immunoglobulins can be used in the Nanobodies of the invention, i.e. as outlined above. For example, the sequence of such a CDR can be determined, synthesized and/or isolated, and inserted into the sequence of a Nanobody of the invention (e.g. so as to replace the corresponding native CDR), all using techniques known per se such as those described herein, or Nanobodies of the invention containing such CDR's (or nucleic acids encoding the same) can be synthesized de novo, again using the techniques mentioned herein.

The invention will now be further described by means of the following non-limiting examples and figures, in which the Figures show:

Monovalent TNFα nanobodies

Figure 1: Sequence alignment of human TNFα nanobodies

Figure 2: Sequence alignment of serum albumin specific TNFα nanobodies

Figure 3: Binding of albumin specific TNFα nanobodies to human serum albumin

Figure 4: Binding of albumin specific TNFα nanobodies to rhesus serum albumin

Figure 5: Binding of albumin specific TNFα nanobodies to mouse serum albumin

Figure 6: Purity of TNFα and serum albumin nanobodies (SDS-PAGE)

Figure 7: Western Blot analysis of TNFα and serum albumin nanobodies

Figure 8: Binding of TNFα nanobodies to human TNFα (ELISA)

Figure 9: Binding of TNFα nanobodies to rhesus TNFα (ELISA)

Figure 10: Receptor-inhibition assay on Enbrel for human TNFα

Figure 11: Receptor-inhibition assay on Enbrel for rhesus TNFα

Figure 12: Binding of TNFα nanobodies to human TNFα (Biacore)

Figure 13: Binding of TNFα nanobodies to rhesus TNFα (Biacore)

Figure 14: Binding of TNFα nanobodies to Protein A (Biacore)

Figure 15: Temperature treatment of TNFα and serum albumin nanobodies (Western Blot)

Figure 16: Stability: temperature treatment of TNFα nanobodies (ELISA)

Figure 17: Temperature treatment of serum albumin nanobodies (Biacore)

Bivalent TNFα nanobodies

Figure 18: Purity of bivalent TNFα nanobodies (SDS-PAGE)

Figure 19: Western Blot analysis of bivalent TNFα nanobodies

Figure 20: Receptor-inhibition assay on Enbrel for bivalent TNFα nanobodies

Figure 21: Stability: temperature treatment of bivalent TNFα nanobodies (ELISA)

Humanised monovalent TNFα nanobodies

Figure 22: Multiple sequence alignment of TNF1 humanised nanobodies

Figure 23: Multiple sequence alignment of TNF2 humanised nanobodies

Figure 24: Multiple sequence alignment of TNF3 humanised nanobodies

Figure 25: Multiple sequence alignment of ALB1 humanised nanobodies

Figure 26: Purity of humanised TNFα and serum albumin nanobodies (SDS-PAGE)

Figure 27: Western Blot analysis of humanised TNFα and serum albumin nanobodies

Figure 28: Binding of humanised TNFα nanobodies to human TNFα

Figure 29: Binding of humanised serum albumin nanobodies to human serum albumin

Figure 30: Stability: temperature treatment of humanised TNFα nanobodies (ELISA)

Trivalent TNFα nanobodies

Figure 31: Purity of trivalent TNFα nanobodies (SDS-PAGE)

Figure 32: Western Blot analysis of trivalent TNFα nanobodies

Figure 33: Stability: temperature treatment of trivalent TNFα nanobodies (ELISA)

Humanised monovalent TNFα nanobodies (second round)

Figure 34: Multiple sequence alignment of TNF1 humanised nanobodies

Figure 35: Multiple sequence alignment of TNF2 humanised nanobodies

Figure 36: Multiple sequence alignment of TNF3 humanised nanobodies

Figure 37: Multiple sequence alignment of ALB1 humanised nanobodies

Figure 38: Purity of humanised TNFα nanobodies (SDS-PAGE)

Figure 39: Western Blot analysis of humanised TNFα nanobodies

Figure 40: Binding of humanised TNFα nanobodies to human TNFα

Figure 41: Stability: temperature treatment of humanised TNFα nanobodies (ELISA)

Figure 42: Analysis of purified TNF60 on Silver stained SDS-PAGE gel (A) Coomassie stained SDS-PAGE gel (B) and in Western blot analysis using anti-NB (C) for detection

Figure 43: Chromatogram of analytical size exclusion of TNF60 on Superdex HR75

Figure 44: Binding of TNF60 to human TNF-alpha

Figure 45:Dose response curve obtained in cytotoxicity assay with human TNF-alpha using Nanobody™ TNF60 in comparison with Enbrel (Etanercept), Humira (Adalimumab) and Remicade (Infliximab)

Figure 46: Dose response curve obtained in cytotoxicity assay with rhesus TNFα using Nanobody™ TNF60 in comparison with Enbrel (Etanercept), Humira (Adalimumab) and Remicade (Infliximab)

Figure 47: Pharmacokinetic profile of TNF60 in mice

Figure 48: Immunogenicity profile of TNF60 in mice

Figure 49: Analysis of purified TNF56-PEG40, TNF56-PEG60, TNF56-biotine, TNF55-PEG40, TNF55-PEG60 and TNF55-biotine on Coomassie stained SDS-PAGE gel

Figure 50: Analysis of purified TNF56-PEG40 on SDS-PAGE gel using Silver stain (A) Coomassie stain (B) and in Western blot analysis using anti-NB (C) for detection

Figure 51: Chromatogram of analytical size exclusion of TNF56-PEG40 on Superdex HR 75

Figure 52: Chromatogram of analytical size exclusion of TNF56-PEG40 on Superdex HR 200

Figure 53: Dose response curve obtained in cytotoxicity assay with human TNFα using Nanobody™ TNF56-PEG40 and the monovalent wild-type Nanobody™ TNF1 in comparison with Enbrel (Etanercept), Humira (Adalimumab) and Remicade (Infliximab)

Figure 54: Dose response curve obtained in cytotoxicity assay with rhesus TNFα using Nanobody™ TNF56-PEG40 in comparison with Enbrel (Etanercept), Humira (Adalimumab) and Remicade (Infliximab)

Figure 55: Pharmacokinetic analysis of pegylated bivalent Nanobody™ TNF56-PEG40 and TNF56-PEG60 after intravenous administration in mice

Figure 56: Pharmacokinetic analysis of pegylated bivalent Nanobody™ 3E-3E-PEG20, pegylated bivalent Nanobody™ 3E-3E-PEG40 and bispecific Nanobody™ 3E-3E-AR1 after intravenous administration in mice

Figure 57: Immunogenicity profile of TNF56-PEG40 and TNF56-PEG60 in mice

Figure 58: Efficacy of TNF60 in the prevention of chronic polyarthritis in mice

Figure 59: Efficacy of TNF60 in therapeutic treatment of chronic polyarthritis in mice

Figure 60: Effect of TNF60 Nanobody™ formatting on efficacy in the prevention of chronic polyarthritis in mice

Figure 61: Sequence alignment of Nanobodies™ PMP1C2, 3E, 1A and 3G

Figure 62: Molecular model of TNF-60

The appended Tables form an integral part of the present specification and are as follows:

Monovalent TNFα nanobodies

Table 8: Sequence listing of TNFα nanobodies

Table 9: Koff values of human TNFα nanobodies

Table 10: Homology of TNFα and serum albumin nanobodies to human germline sequences

Table 11: Expression levels of TNFα and serum albumin nanobodies

Table 12: ELISA binding to human and rhesus TNFα

Table 13: Receptor-inhibition assay of TNFα nanobodies

Table14: Biacore analysis of TNFα nanobodies

Table 15: Binding of TNFα nanobodies to TNFα (K_D-values)

Table 16: Potency of TNFα nanobodies to neutralize human (a) and rhesus (b) TNFα

Table 17: OD280nm of TNFα and serum albumin nanobodies after temperature treatment

Table 18: Potency of TNFα nanobodies after temperature treatment

Bivalent TNFα nanobodies

Table 19: Sequence listing of bivalent TNFα nanobodies and linker sequences

Table 20: Bivalent TNFα nanobody constructs

Table 21: Expression levels of bivalent TNFα nanobodies

Table 22: Receptor-inhibition assay of bivalent TNFα nanobodies

Table 23: Potency of TNFα nanobodies to neutralize human (a) and rhesus (b) TNFα

Table 24: OD280nm of bivalent TNFα nanobodies

Humanised monovalent TNFα nanobodies

Table 25: Sequence listing of humanised monovalent TNFα and serum albumin nanobodies

Table 26: Expression levels of humanised TNFα and serum albumin nanobodies

Table 27: Potency of TNFα nanobodies to neutralize human TNFα

Table 28: OD280nm of humanised TNFα and serum albumin nanobodies

Trivalent TNFα nanobodies

Table 29: Sequence listing of trivalent TNFα nanobodies

Table 30: Trivalent TNFα nanobody constructs

Table 31: Expression levels of trivalent TNFα nanobodies

Table 32: Potency of trivalent TNFα nanobodies to neutralize human TNFα

Table 33: Binding of trivalent nanobodies to serum albumin (K_D-values)

Table 34: OD280nm of trivalent TNFα nanobodies

Humanised monovalent TNFα nanobodies (second round)

Table 35: Sequence listing of second round humanised monovalent TNFα nanobodies

Table 36: Expression levels of humanised TNFα nanobodies

Table 37: Potency of TNFα nanobodies to neutralize human TNFα

Table 38: OD280nm of humanised TNFα nanobodies

Table 39: Comparing bio-activity of nanobodies

Further tables

Table 40: Overview of oligonucleotides used in formatting of trivalent Nanobodies™

Table 41: Overview of oligonucleotides used in cloning of trivalent Nanobodies™

Table 42: EC50 values obtained in cytotoxicity assay using trivalent Nanobody™ TNF60 in comparison with commercial controls (Enbrel, Remicade, Humira)

Table 43: Affinity determination of TNF60 and TNF24 on human serum albumin in Biacore. Nd, not determined.

Table 44: Overview of oligonucleotides used in formatting of bivalent Nanobodies™

Table 45: EC50 values obtained in cytotoxicity assay using bivalent Nanobodies™ in comparison with commercial controls (Enbrel, Remicade, Humira)

Table 46: Results of synovium derived fibroblast studies

Table 47: Results of murine air pouch studies

EXAMPLES

Example 1: Identification of TNFα and serum albumin specific nanobodies

Antagonistic nanobodies were identified using two llamas (*Llama glama*) immunized with human TNFα by 6 injections of 100 μg of the cytokine at weekly intervals. Screening was performed using a competition based assay, in which individual nanobodies were analyzed for their capability to inhibit binding of labeled TNFα to its receptor. The albumin specific nanobodies were identified from a llama immunized with human serum albumin. Screening of individual nanobodies was performed by ELISA using human, rhesus and mouse albumin, yielding a panel of nanobodies cross-reacting with the serum albumin of various species.

Example 2: Sequence analysis of isolated nanobodies

Different classes of nanobodies were identified based on sequence analysis (Figure 1) using a BLOSUM62 scoring matrix and a similarity significance value cut-off of ≥ 60%: Class I (PMP1 C2, PMP1 G11, PMP1 H6), Class II (PMP1 G5, PMP1 H2, PMP3 G2), Class IIb (PMP1 D2), Class III (PMP3 D10, PMP5 F10). Table 8 lists the sequences of these TNFα nanobodies (SEQ ID NOs: 52 to 60).

Based on sequence analysis (Figure 2) different classes of serum albumin nanobodies were identified using the BLOSUM62 scoring matrix and a similarity significance value cut-off of ≥ 60%.Table 8 lists the sequences of these serum albumin nanobodies (SEQ ID NOs: 61 to 67).

Example 3: Biacore analysis

TNFα

Binding of nanobodies to TNFα was characterised by surface plasmon resonance in a Biacore 3000 instrument. TNF from different species was covalently bound to CM5 sensor chips surface via amine coupling until an increase of 250 response units was reached. Remaining reactive groups were inactivated. Nanobody binding was assessed at one concentration (1 in 1,000 diluted). Each nanobody was injected for 4 minutes at a flow rate of 45 µl/min to allow for binding to chip-bound antigen. Binding buffer without nanobody was sent over the chip at the same flow rate to allow spontaneous dissociation of bound nanobody for 4 hours. K_{off}-values were calculated from the sensorgrams obtained for the different nanobodies.

Of each class of nanobodies unpurified proteins were analyzed in Biacore. K_{off} data is listed in Table 9.

Representative nanobodies from each class were retained for further analysis based on k_{off} value. For Class I PMP1C2 (TNF1) was selected; PMP1G5 (TNF2) was selected as representative of Class II; PMP5F10 (TNF3) was selected as representative of Class III.

Serum albumin

Binding was assayed as described above except that 1 in 20 dilutions were used. Figures 3, 4 and 5 illustrate screening of albumin specific TNFα nanobodies versus human, rhesus and mouse serum albumin using unpurified protein.

The nanobodies are ranked according to k_{off}-values, see Table III below:

Class	Human	Rhesus	Mouse
C	PMP6A8	PMP6A8	PMP6B4
C	PMP6B4	PMP6B4	PMP6A8
B	PMP6A6	PMP6A6	PMP6A6
B	PMP6C1	PMP6C1	PMP6C1
A	PMP6G8	PMP6G8	PMP6G8
A	PMP6A5	PMP6A5	PMP6A5
D	PMP6G7	PMP6G7	PMP6G7

Table III

The best k_{off} were obtained for members of family C and family B. Cross-reactivity between mouse, human and rhesus serum albumin was also observed for members of those families. A representative nanobody from class B and C was defined for further analysis: PMP6A6 (ALB1) was selected as representative of Class B and PMP6A8 (ALB2) was selected as representative of Class C.

Example 4: Cloning of monovalent nanobodies in pAX051

Description of Escherichia coli expression vector

pAX051 is a derivative of pUC19. It contains the LacZ promoter which enables a controlled induction of expression using IPTG. The vector has a resistance gene for Ampicillin or Carbenicillin. The multicloning sites harbours several restriction sites of which *Sfi*I and *BstE*II are frequently used for cloning of Nanobodies™. In frame with the NB coding sequence the vector codes for a C-terminal c-myc tag and a (His)6 tag. The signal peptide is the gen3 leader sequence which translocates the expressed Nanobody™ to the periplasm.

The DNA coding for the selected nanobodies TNF1 (PMP1C2), TNF2 (PMP1G5), TNF3 (PMP5F10), ALB1 (PMP6A6) and ALB2 (PMP6A8) was cloned in pAX051 and the construct was transformed to TG1 electrocompetent cells. Clones were analyzed for PCR insert and the nucleotide sequence was determined from 4 positive clones. Glycerol stocks were prepared from clones containing the correct sequence and stored at -80°C.

Example 5: Expression of monovalent nanobodies

A preculture was started by inoculating a single colony of the clone expressing the respective nanobodies at 37°C in Luria Broth, Ampicillin/Carbenicillin (100µg/ml) and 2% glucose overnight. This preculture was used to inoculate. Inoculum is 1% percent (v/v) of the production culture (TB medium + Ampicillin/Carbenicillin + 0.1% Glucose). The production culture is grown at 37°C until an OD600nm of 5-10 is reached and nanobody expression is induced by adding IPTG (1mM final concentration). Protein expression is allowed to continue either for 4h at 37°C or overnight at 28°C, at which point cells are collected by centrifugation and stored as wet cell paste at -20°C.

Preparative periplasmic extracts of the -20°C stored wet cell paste are made by resuspending the pellet in Peri-buffer (50mM NaH_2PO_4, 300mM NaCl, adjusted pH to 8.0), rotating the mixture for 30min at 4°C and centrifuging the mixture using a preparative centrifuge (Sorvall RC-3C *Plus* with H-6000A rotor) to pellet the cells. Supernatant, representing a rough extract of the periplasmic space, is collected for further purification.

The His(6)-tagged nanobodies are purified on Immobilized Metal Affinity Chromatography (IMAC). The TALON resin (Clontech) is processed according to the manufacturer's instructions. The extracts are incubated with the resin for 30 min at RT on a rotator. The resin is washed with PBS and transferred to a column. The packed resin is washed with 15 mM Imidazole. The nanobodies are eluted from the column using 150 mM Imidazole. The eluated fractions are analyzed by spotting on Hybond Membrane and visualization with Ponceau. Fractions containing protein are pooled and dialysed against PBS. Dialysed proteins are collected, filter sterilized, concentration determined and stored in aliquots at -20°C.

<u>Characterisation of monovalent TNFα nanobodies</u>

Example 6: Homology to human germline sequences

The nanobody amino acid sequences were compared to the human germline sequences as represented in Table 10. In order of homology to human sequences the nanobodies rank as follows: TNF1 > TNF2 > TNF3 for the TNFα nanobodies; ALB1 > ALB2 for the serum albumin nanobodies.

Example 7: Expression level

Expression levels were calculated and represented in Table 11. In order of yield the nanobodies rank as follows: TNF1>TNF2>TNF3 for the TNFα nanobodies; ALB1 > ALB2 for the serum albumin nanobodies.

Example 8: SDS-Page analysis

To determine the purity, protein samples were analyzed on a 15% SDS-PAGE gel. 10µl Laemmli sample buffer was added to 10µl (1ug) purified protein, the sample was heated for 10 minutes at 95°C, cooled and loaded on a 15% SDS-PAGE gel. The gel was processed according to general procedures and stained with Coomassie Brilliant Blue (CBB). Figure 6 represents the SDS-PAGE for the TNFα-specific and serum albumin-specific nanobodies.

Example 9: Western Blot analysis

100 ng of purified protein was loaded on the gel. Following SDS-PAGE, proteins were transferred to a nitrocellulose membrane using the Mini Trans-Blot® Electrophoretic Transfer Cell (Biorad). The membrane was blocked overnight in PBS, 1% casein at 4°C.
As all constructs were fused to c-myc tag, mouse monoclonal anti-myc antibody was used as a detection tool. In addition, rabbit polyclonal anti-Nanobody (R23) was used as a detection tool. The blot was incubated for 1h at room temperature with agitation in 1/2000 diluted anti-myc antibody in PBS or 1/2000 anti-Nanobody antibody in PBS, 1% casein. The membrane was washed 5 times in PBS before the secondary antibody was applied (rabbit-anti-mouse IgG alkaline phosphatase conjugate, Sigma, A1902, diluted 1/1000 in PBS or goat anti-rabbit IgG alkaline phosphatase conjugate, Sigma, A8025, 1% casein). After incubation with gentle agitation for 1h at room temperature, the membrane was washed 5 times in PBS. Blots were developed using BCIP/NBT solutions and the reaction was stopped by washing the blot with milliQ water when bands were clearly visible. Figure 7 represents the Western Blot analysis.

Example 10: ELISA binding to human and rhesus TNFα

An ELISA was performed to examine binding to human and rhesus TNFα. A 96-well Maxisorp plate was coated with 2 µg/ml Neutravidin in PBS ON at 4°C. Plates were blocked with 1 % caseine for 2hrs at RT. Biotinylated TNFα (400 ng/ml) was added to the wells and incubated for 1hr at RT. Nanobody samples were diluted starting at 2 µg/ml and using 1 in 3 dilutions. Nanobodies were detected using mouse anti-myc (1/2000 diluted) and rabbit anti-

mouse alkaline phosphatase (1/2000 diluted, Sigma, A1902) and pNPP (2mg/ml) as substrate. Figures 9 and 10 respresent the binding in ELISA to human and rhesus TNFα.

Results are summarized in Table12. TNF1 and TNF3 show binding to both human and rhesus TNFα. TNF2 is binding to human TNFα but is only weakly reactive to rhesus TNFα.

Example 11: Receptor-Inhibition assay

The ability to inhibit receptor-ligand interaction was analyzed for rhesus and human TNFα. A 96-well Maxisorp plate was coated with 2 µg/ml Enbrel in PBS ON at 4°C. Plates were blocked with 1 % Caseine for 2hrs at RT. Nanobody samples were pre-incubated for 30 min at RT with biotinylated TNFα (10ng/ml) starting at a concentration of 5 µg/ml and using 1 in 2 dilutions. Samples were added to the plates and incubated for 1 hr at RT. Biotinylated TNFα was detected using Extravidin alkaline phosphatase (1/2000 diluted) and pNPP (2mg/ml) as substrate. Figures 11 and 12 represent an inhibition ELISA for human and rhesus TNFα. Results are summarized in Table 13. Inhibition of ligand/receptor binding is observed for TNF1 and TNF3 for both human and rhesus TNF, while TNF2 is only inhibiting human TNFα.

Example 12: Biacore analysis

TNFα binding

The analysis was performed as described in Example 3. Figures 13 and 14 illustrate the binding to human and rhesus TNFα via Biacore analysis. Results are summarized in Table 14. Binding experiments in Biacore confirm the ELISA results: cross-reactive binding for TNF1 and TNF3, while TNF2 only significantly binds human TNFα.

Serum albumin

Binding was assayed as described above except that series of different concentrations were used. Each concentration was injected for 4 minutes at a flow rate of 45 µl/min to allow for binding to chip-bound antigen. Binding buffer without analyte was sent over the chip at

the same flow rate to allow for dissociation of bound nanobody. After 15 minutes, remaining bound analyte was removed by injection of the regeneration solution (25 mM NaOH).

From the sensorgrams obtained for the different concentrations of each analyte K_D-values were calculated via steady state affinity when equilibrium was reached.

Results are summarized in Table 15. Cross-reactivity is observed for both ALB1 and ALB2. The highest affinity is observed for ALB2 on human and rhesus TNFα. However, the difference in affinity for human/rhesus versus mouse serum albumin is more pronounced for ALB2 (factor 400), while for ALB1 a difference of a factor 12 is observed.

Example 13: Bio-assay

The TNFα sensitive mouse fibroblast cell line L929s was used for measuring the anti-TNFα activity of the selected nanobodies. At a sufficiently high concentration of TNFα in the medium, i.e. cytotoxic dose, L929s cells undergo necrosis. The inhibition of TNFα interaction with its receptor was determined by pre-incubating a series of antibody dilutions with a cytotoxic concentration of TNFα before adding the mixture to the cells. The presence of actinomycin D in the medium sensitises the cells further to TNFα, resulting in increased sensitivity of the bioassay for free TNFα.

The L929 cells were grown to nearly confluency, plated out in 96-well microtiter plates at 5000 cells per well and incubated overnight. Actinomycin D was added to the cells at a final concentration of 1 μg/ml. Serial dilutions of the nanobodies to be tested were mixed with a cytotoxic concentration of TNFα (final assay concentration is 0.5 ng/ml or 15 IU/ml). After at least 30 minutes of incubation at 37°C, this mixture was added to the plated cells. Plates were incubated for 24 hours at 37°C and 5% CO_2. Cell viability was determined by use of the tetrazolium salt WST-1. Dose-response curves and EC_{50} values were calculated with Graphpad Prism.

The results are summarized in Table 16 for human and rhesus TNFα. Based on their potency to neutralize cytotoxic activity, the molecules are ranked as follows: TNF3>TNF1>TNF2 for human TNFα, and TNF1 = TNF3 > TNF2 for rhesus TNFα.

Example 14: Protein A binding

Figure 14 represents Protein A binding analyzed in Biacore as described in Example 12. Positive binding was obtained for TNF1, TNF2, ALB1. No or weak binding was observed for TNF3 and ALB2.

Example 15: Temperature stability

Samples were diluted at 200 µg/ml and divided in 8 aliquots containing 500 µl. The different vials were incubated each at a given temperature ranging from RT to 90°C. After treatment the samples were cooled down for 2hrs at RT, they were kept at 4°C. Precipitates were removed by centrifugation for 30 min at 14,000 rpm. SN was carefully removed and further analysed.

OD280nm

OD at 280 nm was measured and the concentration was calculated. Results are summarized in Table 17. A decrease in protein content was observed for TNF2 and TNF3 starting at 80°C, while for ALB2 a decrease is observed starting from 70°C.

Western Blot

2 µg of treated protein was separated on a 15% SDS-PAGE and transferred to a nitrocellulose membrane and treated as described above. Detection was performed using polyclonal anti-Nanobody (R23, 1/2000 diluted) and anti-rabbit horse radish peroxidase (DAKO, P0448, 1/2000 diluted). Figure 15 represents the Western Blot analysis. A clear drop in protein concentration was observed for ALB2 treated at 70, 80 and 90°C. Aggregation was still observed for TNF1 treated at 70, 80 and 90°C; for TNF3 treated at 90°C; for ALB1 treated at 90°C, meaning that the SN still contains traces of precipitates which result in a higher OD280nm read-out. This explains why the protein concentration as measured at OD280nm does not decrease for TNF1, TNF3 and ALB1 treated at these higher T.

ELISA

The ELISA to detect binding to human TNFα was essentially performed as described in Example 10. Results are presented in Figure 16. Human TNFα binding is decreased for TNF1, TNF2, TNF3 starting at 80°C.

Bio-assay

The bio-assay was performed as described in Example 13. The results are summarized in Table 18. Potency of the nanobodies is decreased for TNF1 starting at 70°C; for TNF2 and TNF3 starting at 80°C.

Biacore

Binding to human serum albumin was determined as described in Example 12. A fixed concentration was used (1 in 50 diluted). Results are presented in Figure 17. Temperature treatment is not influencing binding to serum albumin for ALB1. The treatment has an effect on the k_{on} for ALB2 starting from T=70°C.

Bivalent nanobodies

Example 16: Formatting of bivalent TNFα specific nanobodies

TNF1, TNF2 and TNF3 were formatted to bivalent nanobodies. As spacer between the two building blocks either a 9AA GlySer linker (Table 19 SEQ ID No: 68) or a 30 AA GlySer linker (Table 19 SEQ ID No: 69) was used. This generated the constructs represented by Table 20. Table 19 lists the sequences of these bivalent TNFα nanobodies (SEQ ID NOs: 70 to 75).

Example 17: Expression of bivalent TNFα specific nanobodies

Expression was performed as described in Example 5. The His(6)-tagged nanobodies were purified on Immobilized Metal Affinity Chromatography (IMAC). The Ni-NTA resin (Qiagen) was processed according to the manufacturer's instructions. The extracts were incubated with the resin and incubated for 30 min at RT on a rotator. The resin was washed with PBS and transferred to a column. The packed resin was washed with PBS (1 in 10 diluted). The column was pre-eluted with 15 mM Imidazole. The nanobodies were eluted from the column using 25 mM Citirc Acid pH=4. The eluated fractions were analyzed by spotting on Hybond Membrane and by visualization with Ponceau. Fractions containing protein were pooled and further purified on Cation exchange followed by size exclusion. Purified proteins were collected, filter sterilized, concentration determined and stored in aliquots at -20°C.

Characterisation of bivalent TNFα specific nanobodies

Example 18: Expression level

Expression levels of the bivalent TNFα nanobodies were calculated and represented in Table 21. The linker has no significant effect on the expression level of the nanobodies.

Example 19: SDS-PAGE

SDS-Page was performed as described in Example 8. Figure 18 shows the result of the SDS-Page.

Example 20: Western Blot

Western Blot analysis was performed as described in Example 9. Figure 19 represents the Western Blot results.

Example 21: Receptor-Inhibition assay

The assay was performed as described in Example 11. Figure 20 and Table 22 represent the results. Enhancement of inhibition of ligand/receptor binding was observed for all bivalent nanobodies compared to the monovalent format.

Example 22: Bio-assay

The assay was performed as described in Example 13. Results are summarized in Table 23. Based on their potency to neutralize cytotoxic activity TNF8, TNF7, TNF9 and TNF5 have a potency in the range of Enbrel.

Example 23: Temperature stability

Samples were analysed as described in Example 15.

OD280 nm

OD at 280 nm was measured and the concentration was calculated. Results ae summarized in Table 24. A decrease in protein content was observed for TNF4 and TNF7 starting at 70°C, while for TNF5, TNF6, TNF8 and TNF9 a decrease was observed starting from 80°C.

Western Blot

Samples were analyzed for the presence of aggregates as described in Example 15.

ELISA

The ELISA to detect binding to human TNFα was essentially performed as described above. Results are presented in Figure 21. Human TNFα binding was decreased for TNF5, TNF6, TNF8 and TNF9 starting at 80°C, for TNF4 and TNF7 starting from 70°C.

Humanised monovalent nanobodies

Example 24: Identification of non-human amino acid positions in TNFα and serum albumin specific nanobodies

Figure 22 (TNF1), Figure 23 (TNF2), Figure 24 (TNF3) and Figure 25 (ALB1) represent multiple sequence alignments (Clustal W 1.7) with DP51, DP53, DP54 and DP29 sequences.

In addition to the amino acid mutations, codon optimization was performed yielding the sequences of Table 25 SEQ ID NOs: 76 to 89 (Nanobodies against TNF-alpha and human serum albumin, respectively).

Example 25: Generation of codon optimised mutants

Oligonucleotides were synthesised spanning the entire sequence of the nanobodies.

Example 26: Expression of bivalent TNFα specific nanobodies

Expression was performed as described in Example 5.

Characterisation of humanised nanobody

Example 27: Expression level

Table 26 represents calculated expression levels. Expression was achieved with yields in the range of 3.5-11.7 mg/ml. Induction time did not influence the yield.

Example 28: SDS-PAGE

SDS-PAGE was performed as described in Example 8. Figure 26 represents the SDS-PAGE gel.

Example 29: Western Blot

Western Blot analysis was performed as described in Example 9. Figure 27 represents the Western Blot results.

Example 30: Bio-assay

The assay was performed as described in Example 13.

The results of the humanised nanobodies are summarized in Table 27. The wildtype nanobodies are included as reference.

Example 31: Biacore

The analysis was performed as described in Example 12. Figure 28 and 31 shows Biacore results.

Example 32: Temperature stability

Samples were analysed as described in Example 15.

OD280 nm

OD at 280 nm was measured and the concentration was calculated. Results are summarized in Table 28.

No significant decrease in protein concentration is observed for the humanised TNF1 nanobodies (TNF13-14). A decrease in protein concentration is observed for humanised TNF2 (TNF15-19) and TNF3 (TNF20-23) starting at 80°C. A decrease in protein concentration is observed for humanized ALB1 (ALB4-5) starting at 70°C and for ALB3 starting at 60°C.

Western Blot

Samples were analyzed for the presence of aggregates as described in Example 15.

ELISA

The ELISA to detect binding to human TNFα was essentially performed as described in Example 15. Results are presented in Figure 30.

Human TNFα binding is comparable for temperature treated WT TNF1 and the humanized TNF13 and 14; for temperature treated WT TNF2 and the humanized TNF15-19; human TNFα binding is decreased for TNF21 and 22, and to a less extent for TNF23, while no effect is observed for TNF20 compared to the temperature treated WT TNF3.

Trivalent TNFα nanobodies

Example 33: Formatting of trivalent TNFα specific nanobodies

TNF1, TNF2, TNF3 and ALB1 were formatted to trivalent nanobodies. As spacer between 2 building blocks either a 9AA GlySer linker (Table 19 SEQ ID No 68) or a 30 AA GlySer linker (Table 19 SEQ ID No 69) was used. This generated the constructs of Table 30. Table 29 lists the sequences of trivalent TNFα nanobodies (SEQ ID NOs: 91 to 94).

Example 34: Expression of trivalent TNFα specific nanobody

Expression was performed as described in Example 5. The His(6)-tagged nanobodies are purified on Immobilized Metal Affinity Chromatography (IMAC). The Ni-NTA resin (Qiagen) is processed according to the manufacturer's instructions. The extracts are incubated with the resin and incubated for 30 min at RT on a rotator. The resin is washed with PBS and transferred to a column. The packed resin is washed with PBS (1 in 10 diluted). Pre-elute with 15 mM Imidazole. The nanobodies are eluted from the column using 25 mM Citirc Acid pH=4. The eluated fractions are analyzed by spotting on Hybond Membrane and visualization with Ponceau. Fractions containing protein are pooled and further purified on Cation exchange followed by size exclusion. Purified proteins are collected, filter sterilized, concentration determined and stored in aliquots at -20°C.

<u>Characterization of trivalent TNFα/SA specific nanobodies</u>

Example 35: Expression level

Expression levels were calculated and represented in Table 31.

Example 36: SDS-PAGE analysis

SDS-PAGE was performed as described in Example 8. Figure 31 represents the SDS-PAGE gel.

Example 37: Western Blot analysis

Western Blot analysis was performed as described in Example 9. Figure 32 represents the Western Blot analysis.

Example 38: Bio-assay

The assay was performed as described in Example 13.

The results of the bivalent nanobodies are summarized in Table 32. Based on their potency to neutralize cytotoxic activity, the molecules are equally potent and comparable to their potency as bivalent molecules.

Example 39: Binding to Human Serum Albumin

Binding was assayed as described above except that series of different concentrations were used. Each concentration was injected for 4 minutes at a flow rate of 45 µl/min to allow for binding to chip-bound antigen. Next, binding buffer without analyte was sent over the chip at the same flow rate to allow for dissociation of bound nanobody. After 15 minutes, remaining bound analyte was removed by injection of the regeneration solution (25 mM NaOH).

From the sensorgrams obtained for the different concentrations of each analyte K_D-values were calculated via steady state affinity when equilibrium was reached.

Results are summarized in Table 33. A decrease in affinity was observed for the formatted ALB1 binder compared to the wild type ALB1. The affinity however is still in the range of 7.2-14 nM.

Example 40: Temperature stability

Samples were analysed as described in Example 15.

OD280 nm

OD at 280 nm was measured and the concentration was calculated. Results are summarized in Table 34. A decrease in protein content is observed for TNF24, TNF27 and TNF28 starting at 60°C, while for TNF25 and TNF26 starting from 70°C.

Western Blot

Samples were analyzed for the presence of aggregates as described in Example 15.

ELISA

The ELISA to detect binding to human TNFα was essentially performed as described above. Results are presented in Figure 33. Human TNFα binding is decreased for TNF24 and TNF27, starting from 60°C and for TNF25, TNF26 and TNF28 starting at 70°C.

Humanised monovalent nanobodies (second round)

Example 41: Identification of non-human amino acid positions in TNFα and serum albumin specific nanobodies

Figure 34 (TNF1), Figure 35 (TNF2), Figure 36 (TNF3) and Figure 37 (ALB1) represent multiple sequence alignments (Clustal W 1.7) with DP51, DP53, DP54 and DP29 sequences. The mutated molecules were expressed and purified as described above, yielding the sequences of Table 35 SEQ ID NOs: 95 to 104 (against TNF-alpha and human serum albumin, respectively).

Characterisation of humanised nanobody

Example 42: Expression level

Table 36 represents calculated expression levels. Expression was achieved with yields in the range of 0.5-2.7 mg/ml.

Example 43: SDS-PAGE

SDS-Page was performed as described in Example 8. Figure 38 represents the SDS-Page gel.

Example 44: Western Blot

Western Blot analysis was performed as described in Example 9. Figure 39 represents the Western Blot results.

Example 45: Bio-assay

The assay was performed as described in Example 13.

The results of the humanised nanobodies are summarized in Table 37. The wildtype nanobodies and first round of humanised nanobodies are included as reference.

Example 46: Biacore

The analysis was performed as described in Example 12. Figure 40 shows Biacore results.

Example 47: Temperature stability

Samples were analysed as described in Example 15.

OD280 nm

OD at 280 nm was measured and the concentration was calculated. Results are summarized in Table 38.

No significant decrease in protein concentration is observed for the humanised TNF1 nanobodies (TNF29-30). A decrease in protein concentration is observed for humanised TNF2 (TNF31-32) and TNF3 (TNF33) starting at 80°C.

Western Blot

Samples were analyzed for the presence of aggregates as described in Example 15.

ELISA

The ELISA to detect binding to human TNFα was essentially performed as described in Example 15. Results are presented in Figure 41.

Human TNFα binding is comparable for WT TNF1 and the humanised TNF29 and TNF30; comparable for WT TNF2 and the humanised TNF31 and TNF32; and also for WT TNF3 and humanised TNF33.

Comparative Example

In this Comparative Example, nine Nanobodies of the invention were compared with three Nanobodies from WO 04/041862, called "V_{HH}#1A" or "1A", "V_{HH}3E" or "3E" and "V_{HH}#3G" or "3G" respectively (SEQ ID NOS:1, 4 and 5 in WO 04/041862). The assay used was the cell based assay using KYM-cells referred to in WO 04/41862 (see for example Example 1, under 3)). The results are mentioned in Table 39 below. As can be seen, the Nanobodies of the invention have an EC50 value in this assay that is 18-fold better than the EC50 value of 3E, the best performing Nanobody according to WO 04/041862.

Example 48: Generation of trivalent bispecific humanized Nanobodies™

Trivalent bispecific Nanobodies were formatted and cloned in the E. coli expression vector pAX054 first and then rescued through PCR and cloned in the pPICZαA expression vector.

Description of Escherichia coli expression vector

pAX54 is a derivative of pUC19. It contains the LacZ promoter which enables a controlled induction of expression using IPTG. The vector has a resistance gene for Ampicillin or Carbenicillin. The multicloning sites harbours several restriction sites of which *Sfi*I and *BstE*II are frequently used for cloning of Nanobodies™. The signal peptide is the gen3 leader sequence which translocates the expressed Nanobody™ to the periplasm.

Description of Pichia pastoris expression vector

pPICZαA contains a pUC-derived origin of replication allowing propagation in *E coli*. It contains the promoter of the *Pichia pastoris AOX1* (alcohol oxidase 1) gene. This 942 bp promoter region (i) allows methanol-inducible, high-level expression of the gene of interest, and (ii) targets plasmid integration to the *AOX1* locus following transformation of Pichia with vector DNA that is linearized within the 5′ *AOX1* promoter region. Note that pPICZα vectors do not contain a yeast origin of replication and that, consequently, transformants can only be isolated if recombination occurs between the plasmid and the Pichia genome. The vector specifies resistance to the antibiotic Zeocin in both *E. coli* and *Pichia pastoris* host cells. The vector incorporates the secretion signal of the *Saccharomyces cerevisiae* α-mating factor allowing for efficient secretion of most proteins to the culture medium. The initiation ATG in the α-factor signal sequence corresponds to the native initiation ATG of the *AOX1* gene. The multicloning site harbours several restriction sites of which *Xho*1/*EcoR1* or *Xho*1/*Not*1 are typically used for fusion of the Nanobody™ coding sequences to the secretion signal. The multicloning site is followed by the *AOX1* transcription termination region. More details on this expression vector can be found on the website of Invitrogen (http://www.invitrogen.com/content/sfs/manuals/ppiczalpha_man.pdf).

Formatting trivalent Nanobodies

Three separate PCR reactions were set up to amplify the N-terminal, the middle and the C-terminal Nanobody™ subunit using the oligo combinations indicated in the WPA-0012. The N-terminal Nanobody™ was amplified using M13_rev/ Rev_9GlySer_L108; the middle Nanobody™ was amplified using For_GlySer/Short and Rev_15BspEI_L108; the C-terminal Nanobody™ was amplified using For_BspEI/M13_for. A PCR reaction of 1 µl plasmid DNA (50-100 ng), 1.5 µl forward primer (10 µM → 300 nM), 1.5 µl reverse primer

(10 µM → 300 nM), 1 µl dNTPs (10 mM → 0.2 mM), 5 µl buffer (10x → 1x), 0.75 µl enzyme (3.5 U/µl → 2.6 U/µl) and 39.25 µl H_2O with a total volume of 50 µl was prepared. Primer sequences are given in Table 40. A PCR program was started with 2 minutes at 94°C. A cycle of 30 seconds at 94°C, 30 seconds at 50°C and 1 minute at 72°C was repeated 30 times and followed by 10 minutes at 72°C. Amplification was checked by separating 5 µl of the PCR reaction on a 2% agarose gel. The PCR product was purified using the QIAquick PCR Purification Kit according to the manufacturer's instructions. One column was used and eluted with 50 µl EB buffer. The N-terminal VHH fragment was prepared by incubating 50 µl DNA and 2 µl *BamHI* (10 U/µl) in the appropriate buffer recommended by the manufacturer at 37°C for 2 hours. Subsequently, 2 µl *SfiI* (10U/µl) was added and the mixture was incubated at 55°C for 2 hours. The middle VHH fragment was prepared by incubating 50 µl DNA and 2 µl *BamHI* (10 U/µl) and 2 µl *BspEI* (10 U/µl) in the appropriate buffer recommended by the manufacturer at 37°C for 2 hours. The C-terminal VHH fragment was prepared by incubating 50 µl DNA with 2 µl *BspEI* (10 U/µl) in the appropriate buffer recommended by the manufacturer at 37°C for 2 hours. Subsequently, 2 µl *BstEII* (10U/µl) was added and the mixture was incubated at 60°C for 1 hours. The previous digestion reactions were separated on a 2% agarose gel. The VHH bands (350-450 bp) were cut out of the gel and the DNA was purified using the QIAquick Gel Extraction Kit according to the manufacturer's instructions. One column (with a maximum of 400 mg agarose gel per column) was used and the bound DNA was eluted with 50 µl EB buffer. DNA concentration was determined by measuring OD_{260} (1 OD unit = 50 µg/ml). A ligation mixture with a final volume of 10 µl containing 100 ng vector pAX54, 12 ng N-terminal VHH, 12 ng middle VHH fragment, 12 ng C-terminal VHH fragment fragment, 1 µl ligation buffer and 1 µl ligase (3U) was prepared and incubated for 2 hours at room temperature. Transformation of *E. coli*, TG1 was performed by using 2 µl of ligation mixture. Colonies are analysed using PCR as described in WPA-0010. Sequence analysis is performed on positive clones. Plasmid preparation was performed using the Qiaprep spin Miniprep kit (Qiagen) according to the manufacturer's instructions and described above. Sequencing was performed at the VIB sequence facility, Antwerp, Belgium.

Amplification of coding DNA

The Nanobody™ coding region cloned in the pAX054 *E. coli* expression vector is rescued through PCR using an appropriate primer pair. To ensure that the Nanobody™ is

expressed with a native N-terminus, the coding region is cloned in frame with the Kex2 cleavage site of the secretion signal. The forward primer fuses the C-terminal part of the secretion signal, up to the *Xho*1 recognition site, to the Nanobody™ coding region. A PCR reaction of 1 µl plasmid DNA (50-100 ng), 1.5 µl forward primer (10 µM → 300 nM), 1.5 µl reverse primer (10 µM → 300 nM), 1 µl dNTPs (10 mM → 0.2 mM), 5 µl buffer (10x → 1x), 0.75 µl enzyme (3.5 U/µl → 2.6 U) and 39.25 µl H_2O with a total volume of 50 µl was prepared. Primer sequences are given in Table 41. A PCR program was started with 2 minutes at 94°C. A cycle of 30 seconds at 94°C, 30 seconds at 50°C and 2 minutes at 72°C was repeated 20 times and followed by 10 minutes at 72°C. Amplification was checked by separating 5 µl of the PCR reaction on a 2% agarose gel. The PCR product was purified using the QIAquick PCR Purification Kit according to the manufacturer's instructions. One column was used and the bound DNA was eluted with 50 µl EB buffer.

Cloning strategy

The DNA fragment coding for the NB as well as the pPICZαA expression vector is digested with the appropriate restriction enzymes (*Xho*I + *Not*I). The insert is obtained by incubating 50 µl PCR product with 2 µl *Xho*I (10 U/µl) and 2 µl *Not*I (10 U/µl) in the appropriate buffer recommended by the manufacturer for 3 hrs at 37°C. Vector is obtained similarly, adapting the amount of restriction enzymes to the amount of plasmid. Both the vector and the NB coding fragment are purified and the DNA concentration is quantified using the BioPhotometer (Eppendorf). The fragment and the acceptor vector are ligated in equimolar ratio's using 1 Unit T4 ligase (Promega) for 30 minutes at room temperature or overnight at 16°C. The DNA (20-30 ng) is transformed to TG1 cells. Colonies are analysed through PCR using the 3'AOX1 R and 5'AOX1 F primers. Sequence analysis is performed on positive clones. TNF30, TNF33 and ALB8 were formatted to trivalent bispecific Nanobodies™. As spacer between the building blocks a 9AA GlySer linker was used.

Transformation P. pastoris

To isolate plasmid DNA, a preculture is started by inoculating a single colony of the clone in 50 ml Luria Broth + Ampicillin or Carbenicillin (100µg/ml) + 2% glucose and incubation at 37°C overnight. Plasmid DNA is prepared using the Plasmid Midi kit (Qiagen) according to the manufacturer's instructions. The DNA is linearized by incubating 30 µg plasmid DNA with 6 µl *BstX*1 (10 U/µl) in the appropriate buffer according to the

manufacturer's instructions for 3 hrs at 45°C. Digested DNA is purified using the PCR Purification kit (Qiagen) according to the manufacturer's instructions. The DNA is concentrated using EtOH precipitation according to standard procedures. X-33 electrocompetent cells are transformed with 10 µg linearized DNA and cells are allowed to grow for 48 hrs on a selective YPD agar plate containing Zeocin (100/250/500 µg/ml). X-33 is a wild type *Pichia pastoris* strain; the strain itself as well as the derived recombinant strains contain the native *AOX1* gene and are able to metabolize methanol (Mut^+).

Clones are screened for expression level by inoculating single colonies in 1 ml BGCM in a 24-well plate and growing them for 48 hrs at 30°C at 120 rpm. Cells are centrifuged and fresh BMCM is added to the cells for growth at 30°C at 120 rpm during 48 hrs. Next, MeOH is added to a final concentration of 0.5 % and cells are grown at 30°C at 120 rpm during 8 hrs, after which MeOH is added again to a final concentration of 0.5 %. Cells are grown overnight at 30°C at 120 rpm. Cells are centrifuged and the supernatant is harvested and analysed in ELISA as described in example 10.

Example 49: Expression and purification of trivalent bispecific humanized Nanobodies™

Production in Pichia pastoris

Composition of buffers, solutions and others can be found on the website of Invitrogen (http://www.invitrogen.com/content/sfs/manuals/ppiczalpha_man.pdf).
A preculture was started by inoculating a single colony from plate in 5 ml YPD. The culture was grown overnight at 180 rpm and 30°C. The next day, the pre-culture was diluted to 50 ml of YPD and grown overnight at 180 rpm and 30°C. Production cultures were started by inoculating the pre-culture to a final OD600nm = 0.04-0.08. Cultures were grown in BGCM for 24 hrs at 30 °C at 180 rpm and centrifuged at 4,500 rpm for 30 minutes. Cells were resuspended in 1/3 of the original volume in BMCM medium with a final OD600 nm = 15-20. Cells were induced with MeOH at regular time points, typically 3 times/day, never exceeding the 1 % MeOH content. After 50 hours of induction the supernatant is harvested.

Purification of Nanobody expressed in Pichia pastoris

Culture supernatant is filtered over a .22 µm filtration membrane Micro filtration (Hydrosart, Sartorius). Sample is concentrated using diafiltration on 10kDa ultra filtration membrane (HydroSart, Sartorius) and concentrated to 0.5-1L.

Nanobodies™ are purified using Protein A affinity chromatography (MabSelect Xtra, GE Healthcare) using PBS as running buffer and Glycine [100mM pH=2.5] for elution. Samples are neutralized using 1.5 M Tris pH=8.8. Nanobodies™ are further processed in Anion Exchange Chromatography (Source 30Q, GE Healthcare). Samples are diluted 10-fold with 10mM Piperazine pH=10.2 and adjusted to pH=10.2 with 1M NaOH and a conductivity of <2mS/cm with MilliQ water.

Nanobodies are processed in Size Exclusion chromatography (Superdex 75pg, Hiload XK26/60, GE Healthcare) and LPS is removed via Anion Exchange Chromatography (Source 30Q, GE Healthcare) by passage through 5ml column, which is sanitized with 1M NaOH and equilibrated in Dulbecco PBS.

To determine the purity, protein samples were analyzed on a 15% SDS-PAGE gel as described in example 8. The gel is processed using the SilverQuest™ according to general procedures described by the manufacturer (Invitrogen). Alternatively, gel is processed using coomassie brilliant blue or in western blot as described in example 8 and 9. Results are given in Figure 42.

Example 50: Characterization of trivalent bispecific humanized Nanobodies™

TNF60 consists of 363 amino acids. The protein has a molecular weight of 38,441 Da. The pI is 8.71. The extinction coefficient at 280 nm is 1.736.

Mass spectrophotometry

The mass of the protein was determined in ESI-MS according to standard procedures. The theoretical mass of TNF60 is 38,441 Da. The protein has 2 S-S bridges which should result in a mass of 38,435 Da in ESI-MS. The mass that was experimentally determined for TNF60 derived from 3 different batches ranges from 38,433 Da to 38,435 Da, differing maximally 0.005% with the theoretical mass.

N-terminal sequencing

N-terminal sequencing was performed by Edman degradation according to standard procedures. N-terminal sequencing showed that the protein sequence for the first 7 amino acids is as follows: EVQLVES. This is consistent with the theoretical protein sequence, which indicates proper N-terminal processing.

Analytical sizing

Samples (100 ug) were analysed on the high resolution Superdex75 column, to characterize the different batches of Nanobody™. Size exclusion chromatography of the Nanobody™ typically yields a symmetrical peak, with a retention time of 11.5 ml on Superdex75. The absorbance is typically recorded at 280, 254 and 214 nm. The 214 nm measurement permits higher detection sensitivity. Analytical sizing in PBS provides a symmetrical peak. No contaminants were observed. The retention time observed for 3 different batches is 11,5-11,55 ml. A representative profile is shown in Figure 43.

Example 51: Binding of TNF60 to human TNFα in ELISA

The functionality of TNF60, i.e. binding to human TNFα was analyzed in ELISA as described in example 10. The results are summarized in Figure 44 and clearly demonstrate a dose-dependent and saturable binding of 2 batches of TNF60 to human TNFα.

Example 52: Functionality in Cell-based assay

The potency to neutralize the cytotoxic activity of TNFα was analyzed in a cell-based assay as described in example 13. The results are summarized in Table 42 and in Figures 45 and 46.

The data show that TNF60 has potency in the range of Enbrel/Etanercept and a 10-fold better potency than Humira/Adalimumab and Remicade/Infliximab.

Example 53: Binding of TNF60 to serum albumin

Binding to human and rhesus serum albumin was analyzed in Biacore as described in example 12. KD, kon and koff values are represented in Table 43. TNF60 is compared to TNF24, which is the trivalent bispecific parent Nanobody™ with wild-type building blocks.

Affinity of TNF60 for human and rhesus serum albumin is similar. Affinity is 2-fold lower as compared to the affinity observed for TNF24 which is the wild type analogue of TNF60. K_{on} is identical for both molecules, but the k_{off} is 2-fold higher for TNF60.

Example 54: Pharmacokinetic and immunogenicity analysis of trivalent bispecific humanized Nanobodies in mice

Animals

DBA1 or BALBc mice were warmed up under an infrared lamp and 200 µl Nanobody™ (100 µg per mouse) was injected intravenously in the tail. Blood samples were obtained at different time points by making a small incision in the tail and collecting the blood in a microtube. Typically, blood was sampled at t=15 min, 2hrs, 4hrs, 6 hrs, 1 day, 2 days, 3 days, 4 days, 7 days, 14 days. Serum was prepared according to standard procedures.

Determination of Nanobody™ concentration in mouse serum

A microtiterplate (NUNC, Maxisorb) was coated with 2 µg/ml neutravidin overnight at 4°C. The plate was washed 5 times with PBS/0.05% Tween-20 and blocked for 2 hours at RT with PBS/1% casein. Biotinylated human TNFα (1/2000 in PBS/0.2% casein; 400 ng/ml) was applied to the wells and incubated for 1hr at RT. The standard reference Nanobody™ was applied starting at a concentration of 5 µg/ml and using 5-fold dilutions in PBS containing 1% mouse plasma. The Nanobodies™ were allowed to bind for 2 hours at RT. The plate was washed 5 times and rabbit polyclonal anti-Nanobody (R23) was applied at a 2000-fold dilution for one hour at RT. After washing of the plate, binding was detected with goat-polyclonal-anti-rabbit-HRP (DAKO) at a 3000-fold dilution for one hour at RT, and stained with ABTS/H_2O_2. The OD405nm was measured.

This first ELISA was used to determine the linear range of the standard reference. In a second ELISA, the standard reference was used at concentrations in this linear range and typically using 2-fold dilutions. In this second ELISA, serum test samples were diluted 100-fold and further 5-fold dilutions were made in 1% mouse plasma, to determine the dilution at which the serum samples provide a read-out in the linear range of the standard curve. In a third ELISA, serum samples are diluted at an appropriate concentration determined in the second ELISA and using 2-fold dilutions for accurate determination of the Nanobody™ concentration in the serum samples.

Experiments were performed to determine the pharmacokinetic profile of TNF60 in mice (n=3). A Cmax value of 103,84 ± 31 µg/ml was reached 15 minutes after administration. The half-life (t1/2β) was determined to be 1.9 days, similar to the half-life of mouse serum albumin, indicating that TNF60 adopts the half-life of serum albumin. Data are presented in Figure 47.

Determination of anti-Nanobody antibodies in mice

Nanobody™ was coated at 5 µg/ml in PBS at 4°C overnight. The plate was washed 5 times with PBS/0.05% Tween-20 and blocked for 2 hours at RT with PBS/1% casein. Serum samples were diluted 100-fold and applied to the wells for incubation during 1 hr at room temperature. Detection was performed using 1000-fold diluted polyclonal rabbit anti-mouse-HRP (DAKO, P0260) and using ABTS as substrate.

Serum samples were diluted 50-fold and analyzed for the presence of mouse anti-TNF60 antibodies. Lack of immunogenicity was demonstrated for TNF60. Data are presented in Figure 48.

Example 55: Generation of bivalent long half-lived humanized Nanobodies™

Description of Pichia pastoris expression vector

See example 48

Formatting bivalent Nanobodies™

Two separate PCR reactions were set up to amplify the N-terminal and the C-terminal Nanobody™ subunit using the procedures as indicated in the WPA-0011. For the amplification of the N-terminal Nanobody™ PiForLong and Rev_30GlySer_L108 were used as primer combination; for the amplification of the C-terminal Nanobody™ For_GlySer and PiRevCys1hum were used or alternatively For_GlySer and PiRevCys2hum, introducing the restriction sites required for formatting and free cysteine residues required for C-terminal modifications.

PCR reaction of 1 µl plasmid DNA (50-100 ng), 1.5 µl forward primer (10 µM → 300 nM), 1.5 µl reverse primer (10 µM → 300 nM), 1 µl dNTPs (10 mM → 0.2 mM), 5 µl buffer (10x → 1x), 0,75 µl enzyme (3.5 U/µl → 2.6 U/µl) and 39.25 µl H_2O with a total volume of 50 µl was prepared. Primer sequences are given in Table 44. A PCR program was started with 2 minutes at 94°C. A cycle of 30 seconds at 94°C, 30 seconds at 50°C and 1 minute at 72°C was repeated 30 times and followed by 10 minutes at 72°C. Amplification was checked by separating 5 µl of the PCR reaction on a 2% agarose gel. The PCR product was purified using the QIAquick PCR Purification Kit according to the manufacturer's instructions. One column was used and eluted with 50 µl EB buffer. The N-terminal VHH fragment was prepared by incubating 50 µl DNA and 2 µl *BamHI* (10 U/µl) and 2 µl *Xho*I (10 U/µl)in the appropriate buffer recommended by the manufacturer at 37°C for 1.5 hours. The C-terminal

VHH fragment was prepared by incubating 50 µl DNA and 2 µl *BamHI* (10 U/µl) and 2 µl *EcoRI* (10 U/µl) in the appropriate buffer recommended by the manufacturer at 37°C for 1 hour. The previous digestion reactions were separated on a 2% agarose gel. The VHH bands (350-450 bp) were cut out of the gel and the DNA was purified using the QIAquick Gel Extraction Kit according to the manufacturer's instructions. One column (with a maximum of 400 mg agarose gel per column) was used and the bound DNA was eluted with 50 µl EB buffer. DNA concentration was determined by measuring OD_{260} (1 OD unit = 50 µg/ml). A ligation mixture with a final volume of 10 µl containing 100 ng vector pPICZαA, linearized with *XhoI*/*EcoRI*, 30 ng N-terminal VHH, 30 ng C-terminal VHH fragment fragment, 1 µl ligation buffer and 1 µl ligase (3U) was prepared and incubated for 1 hour at RT. Transformation of *E. coli*, TG1 was performed by using 2 µl of ligation mixture. Colonies are analysed using PCR as decribed in WPA-0010 but using the AOXIFor/AOXIRev primer combination. Sequence analysis is performed on positive clones. Plasmid preparation was performed using the Qiaprep spin Miniprep kit (Qiagen) according to the manufacturer's instructions and described above. Sequencing was performed at the VIB sequence facility, Antwerp, Belgium.

Transformation of P. pastoris

See example 48.

TNF30 was formatted to bivalent Nanobodies™. As spacer between the 2 building blocks a 30 AA GlySer linker was used. To allow for C-terminal site-specific modifications a free cysteine was introduced, either as the last AA of the Nanobody™ or with an extra spacer consisting of GlyGlyGlyCys.

Example 56: Expression and purification of bivalent long half-lived humanized Nanobodies™

Production in Pichia pastoris

See example 49

Purification of bivalent Nanobodies

The culture medium was made cell-free via centrifugation and 0.22µm filtration. The sterile medium was stored at 4°C until further processed. Low molecular weight

contaminants were reduced via ultra filtration on a 10kDa ultra filtration (UF) membrane (HydroSart Sartocon Slice Cassette, Sartorius) as follows: four liter medium was concentrated to 0.5-1 lit, then diluted with 5lit PBS and again concentrated to 0.5lit. This action was carried out twice.

The retentate of the UF was filtered through nylon 47mm membranes 0,45µm (Alltech #2024).

In a next step bivalent Nanobody™ was captured from the concentrated medium via Protein A affinity purification (using MabSelectXtraTM, GE Healthcare). The column [35X100mm] was equilibrated in PBS and after sample application washed extensively with PBS. TNF56 was eluted with Glycine [100mM, pH=2.5].

The eluted fractions of MabSelectXtraTM were neutralized with Tris [1,5M, pH 8,8] and stored at 4°C. TNF56 was concentrated and purified via AEX (A= 10mM piperazin, pH 10,8 and B=1M NaCl in 50mM Tris, pH 7,5) using Source 30Q (GE Healthcare). To this end the Nanobody™ fractions were diluted with A buffer (10mM piperazin, pH 10,8) to a conductivity of 5mS/cm and the pH was adjusted to 10,8. The column [25X100mm] was equilibrated in A buffer before loading the sample onto the column. TNF56 was eluted with a 5 Column Volume (CV) gradient. The pH of the collected fractions was adjusted to 7.8 using 1M Tris pH=7.8.

Pegylation of bivalent Nanobodies™ expressed in *Pichia pastoris*

Reduction of C-terminal cysteines

Dithiotreitol (DTT, Aldrich Cat 15,046-0) was added to the neutralized fractions to reduce potential disulfide bridges that formed between the carboxy terminal cysteines of the Nanobodies™ (usually around 20%). A final concentration of 10mM DTT and incubation overnight at 4°C was found to be optimal. The reduction was evaluated by analytical size exclusion chromatography (SEC). Therefore 25µl of the reduced Nanobody™ was added to 75µl D-PBS and injected on a Sup75 10/300 GL column equilibrated in Dulbecco's PBS (D-PBS, GibcoTM REF 14190-094).

Non reduced Nanobody™ and DTT was removed by preparative SEC on a Hiload 26/60 Superdex75 prep grade column equilibrated in D-PBS.

The concentration of the reduced Nanobody™ was measured by measuring the Absorbance at 280nm. A Uvikon 943 Double Beam UV/VIS Spectrophotometer (method: see

SOP ABL-0038) was used. The absorption was measured in a wavelength scan 245-330nm. Two Precision cells made of Quartz Suprasil® cells were used (Hellma type No.: 104-QS; light path: 10mm). First the absorption of the blank was measured at 280nm by placing two cells filled by 900µl D-PBS. The sample was diluted (1/10) by adding 100µl of the sample to the first cell. The absorption of the sample was measured at 280nm.

The concentration was calculated with following formula: $\frac{OD_{280}\text{Sample} - OD_{280}\text{blank}}{\varepsilon \times l} \times 10$

For TNF55: ε=1,85

For TNF56: ε=1,83.

PEGylation

To PEGylate Nanobody™ a 5X molar excess of freshly made 1mM PEG40 solution was added to the reduced Nanobody™ solution. (MPEG2-MAL-40K of NEKTAR™ Transforming Therapeutics (2D3YOTO1) Mw = 40,000 g/mol; MPEG2-MAL-60K of NEKTAR™ Transforming Therapeutics (2D3YOVO1) Mw = 60,000 g/mol).

The Nanobody™-PEG mixture was incubated for 1h at room temperature (RT) with gentle agitation and then transferred to 4°C. The PEGylation was evaluated via analytical SEC. Therefore 25µl of the Nanobody™ was added to 75µl D-PBS and injected on a Sup75HR 10/300 column equilibrated in D-PBS. Pegylated Nanobody™ eluted in the range of the exclusion volume of the column (>75kDa).

The PEGylated and non PEGylated Nanobody™ were separated via cation exchange chromatography (CEX, using Source30S, GE Healthcare; A buffer =25mM citric acid pH=4 and B=1M NaCl in PBS). The sample was diluted to a conductivity of <5mS/cm and the pH was adjusted to 4,0. The column [25X100mm] was equilibrated and after sample application washed extensively with A-buffer. Pegylated Nanobody™ was eluted with a 3 CV gradient. The collected Nanobody™ was buffer exchanged to D-PBS by SEC on a Hiload 26/60 Superdex 75 prep grade column equilibrated in D-PBS.

Finally the Nanobody™ was made LPS-free via passage over an anion exchange column (Source30Q). The column (10x100mm) was sanitized overnight in NaOH [1M] and afterwards equilibrated in endotoxin free D-PBS.

Biotinylation

To biotinylate Nanobody™ a 5X Molar excess of biotin (EZ-Link® Maleimide-PO2-Biotin, Pierce #21901) from a 10mM stock solution was added to the reduced Nanobody™

(see 5.5.1). The biotin-Nanobody™ mixture was incubated for 1h at RT with gentle agitation and then stored at 4°C.

The purity of biotinylated Nanobody™ was controlled via analytical SEC. Therefore 25µl of biotinylated Nanobody™ was added to 75µl D-PBS and injected on a Sup75HR 10/300 column equilibrated in D-PBS. From the obtained chromatogram could be concluded that the Nanobody™-biotin needs no further purification: no dimerization of Nanobody™ via an oxidation of free sulfidrils could be detected. A buffer change to D-PBS was done by a passage over a desalting column Sephadex G25 fine (90ml) column.

Finally the Nanobody™-biotin was made LPS-free by passage over an anion exchange column (Source30Q, GE Healthcare). The column (1x10cm) was sanitized overnight in 1M NaOH and then equilibrated in D-PBS.

To determine the purity, protein samples were analyzed on a 15% SDS-PAGE gel as described in example 8 and 49. Results are presented in Figure 49 and 50.

Example 57: Characterization of bivalent long half-lived humanized Nanobodies™

Biochemical characterization

TNF55 consists of 260 amino acids. The protein has a molecular weight of 27,106 Da. The pI is 8.67. The extinction coefficient at 280 nm is 1.850.

TNF56 consists of 264 amino acids. The protein has a molecular weight of 27,365 Da. The pI is 8.67. The extinction coefficient at 280 nm is 1.830.

Mass spectrophotometry

The theoretical mass of TNF55 is 27,106 Da. The TNF55-Biotine protein has 2 S-S bridges and a biotine modification which should result in a mass of 27,627 Da in ESI-MS. The mass that was experimentally determined for TNF55-biotine is 27,627 Da.

The theoretical mass of TNF56 is 27,365 Da. The TNF55-Biotine protein has 2 S-S bridges and a biotine modification which should result in a mass of 27,886 Da in ESI-MS. The mass that was experimentally determined for TNF55-biotine is 27,886 Da.

N-terminal sequencing

N-terminal sequencing of TNF56-PEG40 showed that the protein sequence for the first 7 amino acids is as follows: EVQLVES. This is consistent with the theoretical protein sequence, which indicates proper N-terminal processing.

Analytical sizing

Analytical sizing of TNF56-PEG40 in PBS provides a symmetrical peak. No contaminants were observed. The retention time observed is 8.5 ml on Superdex HR 75 and 10.32 ml on Superdex HR 200. A representative profile is shown in Figures 51 and 52.

Example 58: functionality in cell-based assay

The potency to neutralize the cytotoxic activity of TNFα was analyzed in a cell-based assay. Potency was examined at different concentrations of Nanobody™ as well as of the commercially available Enbrel, Humira and Remicade on a molar base. The higher the EC50 observed the lower the activity of the compound to neutralize TNFα.

The results are summarized in Table 45 and Figures 53 and 54.

The data show an increase in potency for the bivalent Nanobodies™ when compared to the monovalent Nanobody™ TNF1. Potency of TNF55 derivatives is similar to TNF56 derivatives, which is in the range of Enbrel and 10-fold better than Humira and Remicade.

Example 59: Pharmacokinetic and immunogenicity analysis of bivalent long half-lived humanized Nanobodies in mice

See example 54

Experiments were performed in order to examine the half-life of pegylated Nanobodies™ in mice. The half-life of bivalent TNF56-PEG40 was compared to the half-life of TNF56-PEG60. Both Nanobodies™ have comparable half-life of ~ 2days. The results are presented in Figure 55.

In addition, the half-life of pegylated bivalent 3E-3E was explored. The half-life of 3E-3E-PEG20 was compared to the half-life of 3E-3E-PEG40 after intravenous administration of 100 μg of the Nanobodies™. 3E-3E-PEG20 has a half-life of 17 hrs, while 3E-3E-PEG40 has a half-life of 2.1 days, comparable to the half-life of 3E-3E-MSA21. The results are presented in Figure 56.

Serum samples were diluted 100-fold and analyzed for the presence of mouse anti-TNF56-PEG40 or anti-TNF56-PEG60 antibodies. Lack of immunogenicity was demonstrated for both molecules. Data are presented in Figure 57.

Example 60: Efficacy of anti-TNF-α Nanobody TNF60 (TNF60) in prevention of chronic polyarthritis

Transgenic mouse lines carrying and expressing a 3'-modified human tumour necrosis factor (hTNF-alpha, cachectin) transgene were used as a model to study the efficacy of TNF60 (TNF60) in preventing the development of arthritis (EMBO J. 10, 4025-4031). These mice have been shown to develop chronic polyarthritis with 100% incidence at four to seven weeks of age.

From the third week of age, litters of transgenic mice were divided into groups of eight animals. Before initializing the study, the average body weight was calculated for each group. From then on, during the whole study animal weights were recorded once a week for each group.

To test the efficacy of TNF60 in the prevention of chronic polyarthritis, intraperitoneal injections were given twice a week to each animal of a particular group according to the following scheme:

-Group 1 (negative control): phosphate buffered saline (PBS) (formulation buffer)
-Group 2 (Nanobody treatment): TNF60 at a final dose of 30 mg/kg
-Group 3 (Nanobody treatment): TNF60 at a final dose of 10 mg/kg
-Group 4 (Nanobody treatment): TNF60 at a final dose of 3 mg/kg
-Group 5 (1st positive control): Enbrel at a final dose of 30 mg/kg
-Group 6 (1st positive control): Enbrel at a final dose of 10 mg/kg
-Group 7 (2nd positive control): Remicade at a final dose of 30 mg/kg
-Group 8 (2nd positive control): Remicade at a final dose of 10 mg/kg
-Group 9 (2nd positive control): Remicade at a final dose of 3 mg/kg

For each group, dates of injection and injection volumes were noted.

Injections continued for seven weeks. During this period, clinical scores were recorded by observing macroscopic changes in joint morphology for each animal.

At 10 weeks of age, all mice were sacrificed and sera and joints were collected. Sera were stored at -70°C and ankle joints were conserved in formalin.

For selected groups, ankle joints were embedded in paraffin and sectioned. Ankle joint sections were subsequently used for histopathological evaluation of disease progression.

Results are depicted in figure 58.

Example 61: Efficacy of anti-TNF-α Nanobody TNF60 (TNF60) in therapeutic treatment of chronic polyarthritis

Transgenic mouse lines carrying and expressing a 3'-modified human tumour necrosis factor (hTNF-alpha, cachectin) transgene were used as a model to study the efficacy of TNF60 (TNF60) in therapeutic treatment of arthritis (EMBO J. 10, 4025-4031). These mice have been shown to develop chronic polyarthritis with 100% incidence at four to seven weeks of age.

From the sixth week of age, litters of transgenic mice were divided into groups of eight animals. Before initializing the study, the average body weight was calculated for each group. From then on, during the whole study animal weights were recorded once a week for each group.

To test the efficacy of TNF60 in the therapeutic treatment of chronic polyarthritis, intraperitoneal injections were given twice a week to each animal of a particular group according to the following scheme:

-Group 1 (negative control): phosphate buffered saline (PBS) (formulation buffer)
-Group 2 (Nanobody treatment): TNF60 at a final dose of 30 mg/kg
-Group 3 (Nanobody treatment): TNF60 at a final dose of 10 mg/kg
-Group 4 (1st positive control): Enbrel at a final dose of 30 mg/kg
-Group 5 (2nd positive control): Remicade at a final dose of 30 mg/kg

For each group, dates of injection and injection volumes were noted.

Injections continued for seven weeks. During this period, clinical scores were recorded by observing macroscopic changes in joint morphology for each animal.

At 13 weeks of age, all mice were sacrificed and sera and joints were collected. Sera were stored at -70°C and ankle joints were conserved in formalin.

For selected groups, ankle joints were embedded in paraffin and sectioned. Ankle joint sections were subsequently used for histopathological evaluation of disease progression.

Results are depicted in figure 59.

Example 62: Effect of formatting on efficacy of an anti-TNF-α Nanobody in prevention of chronic polyarthritis

Transgenic mouse lines carrying and expressing a 3'-modified human tumour necrosis factor (hTNF-alpha, cachectin) transgene were used as a model to study the efficacy of an anti-TNF-α Nanobody formatted in different ways in the prevention of chronic polyarthritis (EMBO J. 10, 4025-4031). These mice have been shown to develop chronic polyarthritis with 100% incidence at four to seven weeks of age.

From the third week of age, litters of transgenic mice were divided into groups of eight animals. Before initializing the study, the average body weight was calculated for each group. From then on, during the whole study animal weights were recorded once a week for each group.

To study the efficacy of an anti-TNF-α Nanobody in different formats for prevention of chronic polyarthritis, intraperitoneal injections were given twice a week to each animal of a particular group according to the following scheme:

-Group 1 (negative control): phosphate buffered saline (PBS) (formulation buffer)
-Group 2 (Nanobody format 1): TNF60 at a final dose of 10 mg/kg
-Group 3 (Nanobody format 1): TNF60 at a final dose of 2.5 mg/kg
-Group 4 (Nanobody format 1): TNF60 at a final dose of 1 mg/kg
-Group 5 (Nanobody format 2): TNF56-PEG40 at a final dose of 10 mg/kg
-Group 6 (Nanobody format 2): TNF56-PEG40 at a final dose of 1.8 mg/kg
-Group 7 (Nanobody format 2): TNF56-PEG40 at a final dose of 0.7 mg/kg
-Group 8 (Nanobody format 3): TNF56-biot at a final dose of 1.8 mg/kg
-Group 9 (Nanobody format 4): TNF30 at a final dose of 1 mg/kg
-Group 10 (Nanobody format 5): TNF1 at a final dose of 1 mg/kg
-Group 11 (1st positive control): Enbrel at a final dose of 10 mg/kg
-Group 12 (2nd positive control): Remicade at a final dose of 10 mg/kg

For each group, dates of injection and injection volumes were noted.

Injections continued for seven weeks. During this period, clinical scores were recorded by observing macroscopic changes in joint morphology for each animal.

At 10 weeks of age, all mice were sacrificed and sera and joints were collected. Sera were stored at -70°C and ankle joints were conserved in formalin.

For selected groups, ankle joints were embedded in paraffin and sectioned. Ankle joint sections were subsequently used for histopathological evaluation of disease progression.

Results are depicted in figure 60.

Example 63: Pharmacokinetic study of anti-TNF-α Nanobodies TNF60 (TNF60) and TNF56-PEG40 in rhesus monkey

Captive-bred rhesus monkeys (*Macaca mulatta*) are used to determine the pharmacokinetic profile of TNF60 and TNF56-PEG40.

Sixteen animals are used in this study (eight males and eight females) and are divided into four groups (two males and two females per group). All animals weighed approximately 5 kg and are disease-free for at least six weeks prior to use. Sniff® Pri vegetarisch V3994 serves as food. Sixty g/kg b.w. are offered to each monkey. The residue is removed. At regular intervals (at least twice a year) the food is analyzed based on EPA/USA for contaminants by LUFA-ITL. Tap water is offered ad libitum. The animals in each treatment group are housed in a block of several adjacent cages within the monkey unit. The monkeys are kept singly in V_2A steel cages with a size of 90 cm x 82 cm x 96 cm. The room temperature is maintened at 23°C ± 3°C (maximum range) and the relative humidity at 60% ± 20% (maximum range). Deviations from the maximum range caused for example during cleaning procedure are dealt with in SOPs. The rooms are lit and darkened for periods of 12 hours each.

Two groups are infused with TNF60 and two groups are infused with TNF56-PEG40. Intravenous infusions of TNF60 and TNF56-PEG40 (dissolved in PBS) into the vena cephalica of the right or the left arm using indwelling catheters and a TSE infusion pump (see below) are given at a fixed dose of 2 mg/kg.

Four single administrations are performed, separated by a wash-out period of at least fourteen days. After the last administration the follow-up period is at least eight weeks. Two out of the four groups are treated with TNF60 or TNF56-PEG40 in combination with methotrexate (MTX) (dissolved in PBS). Group 2 is treated with TNF60 and MTX; group 4 is treated with TNF56-PEG40 and MTX. MTX is dosed weekly intramuscularly at 0.2 mg/kg. On the administration days, MTX is given approximately 30 minutes prior to administration. Dosing starts at the first Nanobody administration and will continue throughout the eight week wash-out after the fourth dose. There are fourteen single MTX administrations, separated by a wash-out period of at least one week starting at the first test item administration.

Example 64: Synovium-derived fibroblast studies

In this study the ability of the anti-TNF biologicals, ALX0071 and Etanercept, to attenuate TNFα-induced IL-6 production by RA-synovium derived fibroblasts was assessed.

Isolation of synovial fibroblasts

Synovial joint tissue from consenting RA patients was stored in DMEM-based medium with antibiotics at 4°C for up to 96 hours after joint replacement surgery. Synovial cells were isolated from dissected synovium by collagenase digestion at 37 °C for 2 hours. The resultant cell suspension was then washed by a series of centrifugation and resuspension steps and the resultant cells then cultured at 37 °C in DMEM-based culture medium supplemented with 10% FCS (v/v). The resultant fibroblasts were used for the following experiment at either the second or third passage. Cells from four donors were used in individual experiments. Fibroblasts were seeded into 96-well flat bottom polystyrene plates at 1.5 $x10^4$ cells in a final volume of 250 µL of DMEM-based culture medium supplemented with 10% FCS (v/v) per well and cultured overnight.

Stimulation of synovial fibroblasts

Cells were then incubated for 72 hours in DMEM-based culture medium supplemented with TNFα at 50 ng per mL (3 nM (R&D Systems 210-TA/CF) alone or in the presence of increasing doses of ALX0071 (0.575 to 1920 ng per mL; 0.015 to 50 nM) or Etanercept (Wyeth Labs; 3.75 to 11250 ng per mL; 0.025 to 75 nM). The final volume in each well was 250 µL and each assessment was performed in triplicate. After 72 hours, the supernatant media was removed and stored at –40 °C prior to analysis by IL-6 ELISA (R&D Systems). The inhibition of TNF□-induced IL-6 production was determined and IC_{50} values were calculated for both ALX0071 and Etanercept.

Summary of results

Both ALX10071 and Etanercept dose-dependently reduced TNF□-induced IL-6 production by RA synovium derived fibroblasts from all four donors. There was a similar potency between the two reagents under these assay conditions.

Example 65: Murine Air pouch studies

In this study the ability of the anti-TNF biologicals, ALX0071 and Etanercept, to attenuate TNFα-induced cell infiltration in to a murine air pouch was assessed.

Creation of Air Pouch

Air pouches were formed by the sub-cutaneous (s.c.) injection of 2.5mL of sterile air in to the dorsal surface of anaesthetised male C57Bl/6/J mice (25-30g, Harlan). The pouch was re-inflated by injecting 2.5 ml of sterile air 3 days later.

TNFα stimulation

Six days after the initial creation of the air pouch, the animals were anaesthetised and the pouch injected with 1 ml of 0.5% CarboxyMethylCellulose (CMC) vehicle containing 0.1 μg recombinant human TNFα (R & D Systems, 210-TA-050/CF). In three other groups of animals, ALX0071 (0.0625, 0.125 and 0.25 mg/kg) was injected s.c. 19 hours prior to the injection of TNFα. A second three groups of animals were injected (s.c.) with Etanercept (Wyeth Labs, 0.125, 0.25 and 0.5 mg/kg) immediately prior to the injection of TNFα.

24 hours following TNFα injection, mice were culled with a rising concentration of CO_2. Pouches were lavaged with 2 ml of ice cold endotoxin free sterile PBS containing 5 IU/ml heparin. Volumes were recorded and 0.5 ml aliquots were separated for counting of the total white blood cell (WBC) population on a Sysmex XT-Vet cell counter. The mean and standard error of the mean (SEM) total WBC counts for each group were calculated per ml of lavage fluid withdrawn. Statistical analysis was by ANOVA with Kruskal-Wallis post-test on untransformed data.

Summary of results

Both ALX0071 and Etanercept attenuated the TNFα-induced WBC infiltration in to the air pouches (Table). While this attenuation reached statistical significance at both the 0.125 ($P<0.01$) and 0.25 mg/kg ($P<0.05$) ALX0071 dose groups, statistical significance was not observed with any Etanercept dose group.

Table 8

Name	SEQ ID NO	Sequence
PMP1C2(TNF1)	52	QVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTA LYYCARSPSGFNRGQGTQVTVSS
PMP1G11	53	QVQLQESGGGMVQPGGSLRLSCAASGFDFGVSWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKTTLYLQMNSLKPEDTA LYYCARSPSGSFRGQGTQVTVSS
PMP1H6	54	EVQLVESGGGLVQPGGSLRLSCATSGFDFSVSWMYWVRQAPGKGLE WVSEINTNGLITKYVDSVKGRFTISRDNAKNTLYLQMDSLIPEDTA LYYCARSPSGSFRGQGTQVTVSS
PMP1G5(TNF2)	55	QVQLVESGGGLVQAGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKEREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLLMNSLK PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSS
PMP1H2	56	QVKLEESGGGLVQPGDSLRLSCAASGRTFSDYSGYTYTVGWFRQAP GKEREFVARIYWSSGNTYYADSVKGRFTISRDIAKNTVDLLMNNLE PEDTAVYYCAARDGIPTSRSVESYNYWGQGTQVTVSS
PMP3G2	57	AVQLVESGGGLVQPGDSLRLSCAASGRTFSDYSGYTYTVGWFRQAP GKEREFVARIYWSSGNTYYADSVKGRFTISRDIAKNTVDLLMNNLE PEDTAVYYCAARDGIPTSRSVESYNYWGQGTQVTVSS
PMP1D2	58	AVQLVDSGGGLVQAGGSLRLSCAASGRTFSAHSVYTMGWFRQAPGK EREFVARIYWSSANTYYADSVKGRFTISRDNAKNTVDLLMNCLKPE DTAVYYCAARDGIPTSRSVEAYNYWGQGTQVTVSS
PMP3D10	59	QVQLVESGGGLVQAGGSLSLSCAASGRSFTGYYMGWFRQAPGKERQ LLASISWRGDNTYYKESVKGRFTISRDDAKNTIYLQMNSLKPEDTA VYYCAASILPLSDDPGWNTNWGQGTQVTVSS
PMP5F10(TNF3)	60	EVQLVESGGGLVQAGGSLSLSCSASGRSLSNYYMGWFRQAPGKERE LLGNISWRGYNIYYKDSVKGRFTISRDDAKNTIYLQMNRLKPEDTA VYYCAASILPLSDDPGWNTYWGQGTQVTVSS
PMP6A8(ALB2)	61	AVQLVESGGGLVQGGGSLRLACAASERIFDLNLMGWYRQGPGNERE LVATCITVG.DSTNYADSVKGRFTISMDYTKQTVYLHMNSLRPEDT GLYYCKIRRTWHSELWGQGTQVTVSS
PMP6B4	62	EVQLVESGGGLVQEGGSLRLACAASERIWDINLLGWYRQGPGNERE LVATITVG.DSTSYADSVKGRFTISRDYDKNTLYLQMNSLRPEDTG LYYCKIRRTWHSELWGQGTQVTVSS
PMP6A6(ALB1)	63	AVQLVESGGGLVQPGNSLRLSCAASGFTFRSFGMSWVRQAPGKEPE WVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTA VYYCTIGGSLSRSSQGTQVTVSS
PMP6C1	64	AVQLVDSGGGLVQPGGSLRLSCAASGFSFGSFGMSWVRQYPGKEPE WVSSINGRGDDTRYADSVKGRFSISRDNAKNTLYLQMNSLKPEDTA EYYCTIGRSVSRSRTQGTQVTVSS
PMP6G8	65	AVQLVESGGGLVQPGGSLRLTCTASGFTFRSFGMSWVRQAPGKDQE WVSAISADSSTKNYADSVKGRFTISRDNAKKMLYLEMNSLKPEDTA VYYCVIGRGSPSSPGTQVTVSS

PMP6A5	66	QVQLAESGGGLVQPGGSLRLTCTASGFTFGSFGMSWVRQAPGEGLE WVSAISADSSDKRYADSVKGRFTISRDNAKKMLYLEMNSLKSEDTA VYYCVIGRGSPASQGTQVTVSS
PMP6G7	67	QVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMYWVRVAPGKGLE RISRDISTGGGYSYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDT ALYYCAKDREAQVDTLDFDYRGQGTQVTVSS
NC55TNF_S1C4	105	EVQLVESGGGLVQAGDSLRLSCAASQIIFGSHVAAWFRQAPGRERE FVAEIRPSGDFGPEGEFEHVTASLKGRFTIAKNSVDNTVYLQMNSL KPEDTAVYYCAAAPYRGGRDYRWEYEYEYWGQGTQVTV
NC55TNF_S1C3	106	EVQLVESGGGLVQPGGSLRLSCKNAGSTSNAYATGWFRRAPGKERE FVAGIQWSGGDAFYRNSVKGRFRITRDPDNTVYLQMNDLKPEDTAI YYCAQKLSPYYNDFDSSNYEYWGQGTQVTV
NC55TNF_S2C1	107	EVQLVESGGDLVQPGGSLRLSCAVSGQLFSTNDVGWYRRAPGKQRE LVATITDDGTTDYGDDVKGRFVISREGEMVYLEMNSLKPEDTAVYY CNINRLRSTWGIRYDVWGQGTQVTVSS
NC55TNF_S2C5	108	EVQLVESGGGLVQPGGSLRLSCVVSGFTFSTTSMTWVRQAPGKFEE WVSFINSDGSSTTYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTA MYYCGRRGYGRDRSKGIQVTVAS
NC55TNF_S3C7	109	EVQLVESGGGTVQAGDSLRLSCAASGRSFSSVAMGWFRQAPGKQRE FLAGVGYDGSSIRYAESVKGRFTIARGNRESTVFLQMENLKPEDTA VYFCTAEPIGAYEGLWTYWGQGTQVTVSS
NC55TNF_S3C1	110	XXXXVESGGGLMQPGGSLKLSCAASGFMFSDSAMGWFRQAPGKERE FVATISWNGGSSSYADFVKGRFTISRDNAKNTVYLQMNGLTPQDTA IYYCAGSYSNGNPHRFSQYQYWGQGTQVTVSS
NC55TNF_BMP1B2	111	EVQLVESGGGLVQAGGSLRLSCAASGRTFGTYAMGWFRQAPGKERE FVAAISWGGGSIVYAESAKGRFTISRDNAKXTMYLQMDSLKPEDTA VYYCAAANNIATLRQGSWGQGTQVTVSS
NC55TNF_BMP1D2	112	EVQLVESGGELVQAGGSLKLSCTASGRNFVTYAMSWFRRAPGKERE FVASISWSGDTTYYSNSVKGRFTVSRDNGKNTAYLRMNSLKPEDTA DYYCAVVQVIDPSWSGVNLDDYDYLGSGTQVTVSS
NC55TNF_BMP1E2	113	EVQLVESGGRLVQPGGSLRLSCKNAGSTSNAYATGWFRRAPGKERE FVAGIQWSGGDAFYRNSVKGRFRITRDPDNTVYLQMNDLKPEDTAI YYCAQKLSPYYNDFDSSNYEYWGQGTQVTVSS
NC55TNF_BMP1G2	114	EVQLVESGGGLVQPGGSLRLSCAASATISSIVMLGWYRQAPGKQRE WVASITIGSRTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAV YFCNAVPPRDDYWGQGTQVTVSS
NC55TNF_BMP2A2	115	EVQLVESGGGLVQAGGSLRLSCAASGQTSSSYDMGWFRQAPGEGRE FVARISGSDGSTYYSDRAKDRFTISRDNTKNMVYLQMDRLKPDDTA VYYCRVPRYENQWSSYDYWGQGTQVTVSS
NC55TNF_BMP2C2	116	EVQLVESGGGLVQPGGSLRLSCAASGSTFSTYDMSWVRQAPGKGLE WVSGIDSGGGSPMYVDSVKGRFTVSRDNAKNTLYLQMNSLKPEDTA VYYCAKFSTGADGGSWYWSYGMDSWGKGTQVTVSS
NC55TNF_BMP2F2	117	EVQLVESGGGLVQAGDSLRLSCEASERSSNRYNMAWFRQAPGKERE FLARVDVSGGNTLYGDSVKDRFTVSRINGKNAMYLQMNNLKPEDTA IYYCAAGGWGTTQYDYDYWGQGTQVTVSS
NC55TNF_NC10	118	EVQLVESGGGLVQPGGSLRLSCVCVSSGCTFSAYSMTWVRQAPGKA EEWVSFINSDGSSTTYADSVNGRFKISRDNAKKTLYLQMNSLGPED TAMYYCQRRGYALDRGQGTQVTVSS
NC55TNF_NC11	119	EVQLVESGGGLVQAGDSLTLSCASSGRGFYKNAMGWFRQPPGKERE FVASIKWNGNNTYYADSVRGRFTISRGNAKNTENTVSLQMNSLKPE DTADYYCAADSSHYSYVYSKAYEYDYWGQGTQVTVSS
NC55TNF_NC1	120	EVQLVESGGGLVQPGGSLRLSCVFSGFAFSASSMAWVRQAPGKYEE WVSFINSDGSSTTYADSVQGRFTISRDNAKNTLYLQMNSLKSEDTA MYYCGRRGYGRDRSQGIQVTVSS
NC55TNF_NC2	121	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAMGWFRQAPGKERE FVAAISWSGTITNYADSVKGRFTISRDNGKNTVHLQMNSLKPEDTA VYHCAVVQPYSGGDYYTGVEEYDYWGXGTQVTVSS

NC55TNF_NC3	122	EVQLVESGGGLVQPGGSLRLSCVVSGFTFSATSMTWVRQAPGKAEE WVSFINSDGSSTTYADSVKGRFTISRDNAKNTLYLQMDDLQSEDTA MYYCGRRGYGRDRSRGIQVTVSS
NC55TNF_NC5	123	EVQLVESGGGLVQAGGSLRLSCAASGGAFSNYDVGWFRQAPGEGRE IVARISGSGDSTYSSNRAKGRFTISRDNAKNTVYLQMNSLKREDTA VYYCRAARYNGTWSSNDYWGQGTQVTVSS
NC55TNF_NC6	124	EVQLVESGGGLVQPGGSLRLSCECVSSGCTFSAYSMTWVRQAPGKA EEFVSFINSDGSSTTYANSVNGRFKISRDNAKKTLYLQMNSLGPED TAMYYCQRRGYALDRGQGTQVTVSS
NC55TNF_NC7	125	QVQLVESGGGLVQAGGSLRLSCTASGQTSSTADMGWFRQPPGKGRE FVARISGIDGTTYYDEPVKGRFTISRDKAQNTVYLQMDSLKPEDTA VYYCRSPRYADQWSAYDYWGQGTQVTVSS
NC55TNF_NC8	126	EVQLVESGGGLVQPGGSLRLSCVVSGFTFSTTSMTWVRQAPGKFEE WVSFINSDGSSTTYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTA MYYCGRRGYGRDRSKGIQVTVSS
NC55TNF_S2C2	127	EVQLVESGGGLVQPGGSLRLSCVASASGVKVNDMGWYRQAPGKERE LVATITDDGRTNYEDFAKGRFTISRDNAKNTVYLQMNSLLPEDTAV YYCNARTYWAHLPTYWGQGTQVTVSS
NC55TNF_S1C6	128	EVQLVESGGGLVQAGGSLRLSCAASGRSFGSVAMGWFRQAPGKERE FVAAIGYDGNSIRYGDSVKGRFTISRDNIKNTMYLEMENLNADDTA RYLCAAEPLARYEGLWTYWGQGTQVTVSS
NC55TNF_S3C2	129	EVQLVESGGGLVQAGASLRLSCTTSTRTNDRFNMAWFHQAPGKDRE FVSRIDVAGYNTAYGDFVKGRFTVSRDSAENTVVLQMNSLRPEDTG VYYCAAGGWGISQSDYDLWGQGTQVTVSS

Table 9

nanobody	Class	Estimated koff (1/s)
PMP5 F10	III	2,63E-04
PMP1 G5	II	3,59E-04
PMP1 C2	I	4,39E-04
PMP1 G11	I	1,15E-03
PMP1 H6	I	2,14E-03
PMP1 H2	II	3,65E-03
PMP3 G2	II	1,09E-02

Table 10

Nanobody	**Germline sequence**	**#AA differences / total #AA**	**% AA identity**
ALB1	DP51/DP53	13/87	85.1
ALB2	DP54	26/87	70.2
TNF1	DP51/DP53	6/87	93.2
TNF2	DP54	16/87	81.7
TNF3	DP29	18/87	79.4

Table 11

Nanobody	**Induction time**	**Yield (mg/L)**
ALB1	short/37°C	18
ALB2	short/37°C	4
TNF1	short/37°C	8.3
TNF2	short/37°C	5
TNF3	short/37°C	0.8

Table 12

	50% binding (ng/ml)	
ID	**Human TNFα**	**Rhesus TNFα**
TNF1	12	12
TNF2	20	>3000
TNF3	18	16

Table 13

	50% inhibition (ng/ml)	
ID	**Human TNFα**	**Rhesus TNFα**
TNF1	530	220
TNF2	3500	>5000
TNF3	100	100

Table 14

Human TNFα	Kd (1/s)
TNF1	1,05E-03
TNF2	1,33E-03
TNF3	3,02E-04

Table 15

		Human albumin	Rhesus albumin	Mouse albumin
ALB1	**KD (nM)**	0,57	0,52	6,5
	ka (1/Ms)	1,11E+06	1,05E+06	1,11E+06
	kd (1/s)	6,30E-04	5,46E-04	7,25E-03
ALB2	**KD (nM)**	0,092	0,036	15,7
	ka (1/Ms)	8,15E+05	1,94E+06	1,95E+05
	kd (1/s)	7,52E-05	7,12E-05	3,07E-03

Table 16

assay : ***L929s + Act D (5000c/w)***

TNF : ***human* TNFα @ *0.5ng/ml***

		EC_{50} in nM			relative potency	
Nanobody		**mean**	**stdev**	**#**	**mean**	**stdev**
TNF1	1C2	0.707	0.265	14	0.015	0.007
TNF2	1G5	1.412	0.622	14	0.007	0.002
TNF3	5F10	0.224	0.133	14	0.048	0.019
Enbrel		0.009	0.005	45	1.002	0.011
Humira		0.079	0.043	39	0.097	0.069
Remicade		0.083	0.037	45	0.103	0.058

assay : ***L929s + Act D (5000c/w)***

TNF : ***rhesus* TNFα @ *0.5ng/ml***

		EC_{50} in nM			relative potency	
Nanobody		**mean**	**stdev**	**#**	**mean**	**stdev**
TNF1	1C2	0.693	0.305	9	0.015	0.009
TNF2	1G5	>50		9		
TNF3	5F10	0.602	0.283	9	0.017	0.010
Enbrel		0.009	0.003	7	1	0.000
Humira		0.071	0.025	8	0.103	0.059
Remicade		>6.7		7		

Table 17

%	**Untreated**	**RT**	**37°C**	**50°C**	**60°C**	**70°C**	**80°C**	**90°C**
TNF1	100	98	98	98	98	95	92	90
TNF2	100	99	100	99	97	96	63	50
TNF3	100	96	97	98	96	94	75	70
ALB1	100	101	102	101	100	64	94	90
ALB2	100	100	102	100	100	28	8	17

Table 18

nanobody	temp in °C	EC_{50} in nM	#	relative potency
TNF1	control	0,916	1	0,013
92#2302nr1.TNF1	RT	0,873	1	0,014
	37	0,901	1	0,013
	50	0,908	1	0,013
	60	0,891	1	0,013
	70	1,218	1	0,010
	80	2,655	1	0,004
	90	5,797	1	0,002
TNF2	control	2,500	1	0,005
92#2302nr2.TNF2	RT	2,165	1	0,005
	37	2,212	1	0,005
	50	2,241	1	0,005
	60	1,782	1	0,007
	70	2,487	1	0,005
	80	2,818	1	0,004
	90	6,135	1	0,002
TNF3	control	0,278	1	0,043
92#2302nr3.TNF3	RT	0,289	1	0,041
	37	0,295	1	0,040
	50	0,290	1	0,041
	60	0,281	1	0,042
	70	0,293	1	0,040
	80	0,576	1	0,021
	90	0,861	1	0,014

Table 19

Name	SEQ ID NO	Sequence
GS9	68	GGGGSGGGS
GS30	69	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGS
TNF1-GS9-TNF1(TNF4)	70	QVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTA LYYCARSPSGFNRGQGTQVTVSSGGGGSGGGSQVQLVESGGGLVQP GGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKY PDSVKGRFTISRDNAKNTLYLQMNSLKPEDTALYYCARSPSGFNRG QGTQVTVSS
TNF2-GS9-TNF2(TNF5)	71	QVQLVESGGGLVQAGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKEREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLLMNSLK PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSSGGGGSGGGS QVQLVESGGGLVQAGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKEREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLLMNSLK PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSS
TNF3-GS9-TNF3(TNF6)	72	EVQLVESGGGLVQAGGSLSLSCSASGRSLSNYYMGWFRQAPGKERE LLGNISWRGYNIYYKDSVKGRFTISRDDAKNTIYLQMNRLKPEDTA VYYCAASILPLSDDPGWNTYWGQGTQVTVSSGGGGSGGGSEVQLVE SGGGLVQAGGSLSLSCSASGRSLSNYYMGWFRQAPGKERELLGNIS WRGYNIYYKDSVKGRFTISRDDAKNTIYLQMNRLKPEDTAVYYCAA SILPLSDDPGWNTYWGQGTQVTVSS
TNF1-GS30-TNF1(TNF7)	73	QVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTA LYYCARSPSGFNRGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGG GSGGGGSQVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQ APGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNS LKPEDTALYYCARSPSGFNRGQGTQVTVSS
TNF2-GS30-TNF2(TNF8)	74	QVQLVESGGGLVQAGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKEREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLLMNSLK PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSSGGGGSGGGG SGGGGSGGGGSGGGGSGGGGSQVQLVESGGGLVQAGGSLRLSCAAS GRTFSEPSGYTYTIGWFRQAPGKEREFVARIYWSSGLTYYADSVKG RFTISRDIAKNTVDLLMNSLKPEDTAVYYCAARDGIPTSRSVGSYN YWGQGTQVTVSS
TNF3-GS30-TNF3(TNF9)	75	EVQLVESGGGLVQAGGSLSLSCSASGRSLSNYYMGWFRQAPGKERE LLGNISWRGYNIYYKDSVKGRFTISRDDAKNTIYLQMNRLKPEDTA VYYCAASILPLSDDPGWNTYWGQGTQVTVSSGGGGSGGGGSGGGGS GGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLSLSCSASGRSLSN YYMGWFRQAPGKERELLGNISWRGYNIYYKDSVKGRFTISRDDAKN TIYLQMNRLKPEDTAVYYCAASILPLSDDPGWNTYWGQGTQVTVSS
TNF30-30GS-TNF30-C (TNF 55)	419	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTA VYYCARSPSGFNRGQGTLVTVSSggggsggggsggggsggggsggg gsggggsEVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQ APGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNS LRPEDTAVYYCARSPSGFNRGQGTLVTVSC
TNF30-30GS-TNF30-gggC (TNF56)	420	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTA VYYCARSPSGFNRGQGTLVTVSSggggsggggsggggsggggsggg gsggggsEVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQ APGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNS LRPEDTAVYYCARSPSGFNRGQGTLVTVSSgggC

Table 20

ID	Format	Linker
TNF4	TNF1-TNF1	9 AA GlySer
TNF5	TNF2-TNF2	9 AA GlySer
TNF6	TNF3-TNF3	9 AA GlySer
TNF7	TNF1-TNF1	30 AA GlySer
TNF8	TNF2-TNF2	30 AA GlySer
TNF9	TNF3-TNF3	30 AA GlySer

Table 21

Nanobody™	Induction time	Yield (mg/L)
TNF4	ON/28°C	3.2
TNF5	short/37°C	5.5
TNF6	short/37°C	1.19
TNF7	ON/28°C	2.7
TNF8	short/37°C	6.6
TNF9	ON/28°C	1.3

Table 22

	50% inhibition (ng/ml)
ID	Human TNFα
TNF4	13
TNF5	6.3
TNF6	30
TNF7	16
TNF8	23
TNF9	18

Table 23

assay : ***L929s + Act D (5000c/w)***

TNF : ***human TNFa @ 0.5ng/ml***

	EC_{50} in nM			relative potency	
Nanobody	**mean**	**stdev**	**#**	**mean**	**stdev**
TNF4	0.236	0.049	4	0.033	0.012
TNF5	0.020	0.010	9	0.566	0.275
TNF6	0.078	0.047	8	0.179	0.168
TNF7	0.013	0.005	8	0.673	0.211
TNF8	0.007	0.002	2	1.240	0.137
TNF9	0.012	0.005	6	0.729	0.242
Enbrel	0.009	0.005	45	1.002	0.011
Humira	0.079	0.043	39	0.097	0.069
Remicade	0.083	0.037	45	0.103	0.058

assay : ***L929s + Act D (5000c/w)***

TNF : ***rhesus TNFa @ 0.5ng/ml***

	EC_{50} in nM			relative potency	
Nanobody	**mean**	**stdev**	**#**	**mean**	**stdev**
TNF4	0.141	0.025	4	0.065	0.015
TNF5	35.000	16.000	5	0.000	0.000
TNF6	0.398	0.074	6	0.024	0.003
TNF7	0.011	0.005	4	0.860	0.142
TNF8	1.026	0.444	2	0.010	0.001
TNF9	0.038	0.012	4	0.249	0.032
Enbrel	0.009	0.003	7	1.000	0.000
Humira	0.071	0.025	8	0.103	0.059
Remicade	>6.7		7		

Table 24

%	untreated	RT	37°C	50°C	60°C	70°C	80°C	90°C
TNF4	100	99	99	99	98	55	34	17
TNF5	100	99	101	99	98	92	26	22
TNF6	100	103	104	103	105	99	7	7
TNF7	100	100	100	98	96	66	33	40
TNF8	100	99	100	99	100	89	11	8
TNF9	100	101	101	101	101	99	17	18

Table 25

Name	SEQ ID NO	Sequence
TNF13(TNF1 HUM1)	76	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTA VYYCARSPSGFNRGQGTQVTVSS
TNF14(TNF1 HUM2)	77	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTA LYYCARSPSGFNRGQGTLVTVSS
TNF15(TNF2 HUM1)	78	EVQLVESGGGLVQPGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKGREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLQMNSLK PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSS
TNF16(TNF2 HUM2)	79	EVQLVESGGGLVQPGGSLRLSCAASGFTFSEPSGYTYTIGWFRQAP GKGREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLQMNSLK PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSS
TNF17(TNF2 HUM3)	80	EVQLVESGGGLVQPGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKGREFVARIYWSSGLTYYADSVKGRFTISRDNAKNTVDLQMNSLK PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSS
TNF18(TNF2 HUM4)	81	EVQLVESGGGLVQPGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKGREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLQMNSLR PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSS
TNF19(TNF2 HUM5)	82	EVQLVESGGGLVQPGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKGREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLQMNSLK PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTLVTVSS
TNF20(TNF3 HUM1)	83	EVQLVESGGGLVQPGGSLRLSCAASGRSLSNYYMGWFRQAPGKGRE LLGNISWRGYNIYYKDSVKGRFTISRDDSKNTIYLQMNSLKPEDTA VYYCAASILPLSDDPGWNTYWGQGTQVTVSS
TNF21(TNF3 HUM2)	84	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYYMGWFRQAPGKGRE LLGNISWRGYNIYYKDSVKGRFTISRDDSKNTIYLQMNSLKPEDTA VYYCAASILPLSDDPGWNTYWGQGTQVTVSS
TNF22(TNF3 HUM3)	85	EVQLVESGGGLVQPGGSLRLSCAASGRSLSNYYMGWFRQAPGKGRE LLGNISWRGYNIYYKDSVKGRFTISRDDSKNTIYLQMNSLKTEDTA VYYCAASILPLSDDPGWNTYWGQGTQVTVSS
TNF23(TNF3 HUM4)	86	EVQLVESGGGLVQPGGSLRLSCAASGRSLSNYYMGWFRQAPGKGRE LLGNISWRGYNIYYKDSVKGRFTISRDDSKNTIYLQMNSLKPEDTA VYYCAASILPLSDDPGWNTYWGQGTLVTVSS
ALB3(ALB1 HUM1)	87	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKEPE WVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTA VYYCTIGGSLSRSSQGTQVTVSS
ALB4(ALB1 HUM2)	88	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSFGMSWVRQAPGKEPE WVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTA VYYCTIGGSLSRSSQGTQVTVSS
ALB5(ALB1 HUM3)	89	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGLE WVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTA VYYCTIGGSLSRSSQGTQVTVSS

Table 26

Nanobody	Induction time	Yield (mg/L)
TNF13	ON/28°C	8.8
TNF14	ON/28°C	7
TNF15	Short/37°C	7.6
TNF16	Short/37°C	8.7
TNF17	Short/37°C	7.2
TNF18	Short/37°C	4.8
TNF19	Short/37°C	8
TNF20	ON/28°C	3.5
TNF21	ON/28°C	7.5
TNF22	ON/28°C	6
TNF23	ON/28°C	2.8
ALB3	ON/28°C	11.8
ALB4	ON/28°C	9
ALB5	ON/28°C	11.7

Table 27

assay : ***L929s + Act D (5000c/w)***

TNF : ***human TNFa @ 0.5ng/ml***

	EC_{50} in nM			**relative potency**	
Nanobody	**mean**	**stdev**	**#**	**mean**	**stdev**
TNF1	**0.707**	**0.265**	**14**	**0.015**	**0.007**
TNF13	0.988	0.014	3	0.014	0.003
TNF14	0.981	0.007	3	0.014	0.003
TNF2	**1.412**	**0.622**	**14**	**0.007**	**0.002**
TNF15	1.669	1.253	4	0.002	0.000
TNF16	1.898	0.192	4	0.005	0.001
TNF17	3.023	0.562	4	0.001	0.001
TNF18	1.508	0.481	4	0.004	0.001
TNF19	2.191	0.941	4	0.001	0.001
TNF3	**0.224**	**0.133**	**14**	**0.048**	**0.019**
TNF20	0.380	0.080	3	0.035	0.005
TNF21	0.889	0.019	3	0.015	0.003
TNF22	0.303	0.005	3	0.044	0.011
TNF23	0.3	0.011	3	0.044	0.011
Enbrel	0.009	0.005	45	1.002	0.011
Humira	0.079	0.043	39	0.097	0.069
Remicade	0.083	0.037	45	0.103	0.058

Table 28

%	Untreated	RT	37C	50C	60C	70C	80C	90C
TNF13	100	104	99	98	99	84	93	93
TNF14	100	98	101	95	99	96	99	90
TNF15	100	100	91	99	95	90	59	46
TNF16	100	97	102	101	94	101	58	48
TNF17	100	102	98	100	90	90	69	59
TNF18	100	100	101	97	91	93	63	50
TNF19	100	102	111	98	92	91	60	49
TNF20	100	94	93	93	93	92	85	67
TNF21	100	98	99	101	98	96	36	40
TNF22	100	102	101	105	99	93	25	31
TNF23	100	98	97	99	97	98	87	55
ALB3	100	100	99	98	25	18	60	62
ALB4	100	100	100	100	99	29	61	55
ALB5	100	100	100	99	94	32	61	48

Table 29

Name	SEQ ID NO	Sequence
TNF1-9GS-ALB1-9GS-TNF1(TNF24)	90	QVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTA LYYCARSPSGFNRGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQP GNSLRLSCAASGFTFRSFGMSWVRQAPGKEPEWVSSISGSGSDTLY ADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAVYYCTIGGSLSRSS QGTQVTVSSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGFT FSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDN AKNTLYLQMNSLKPEDTALYYCARSPSGFNRGQGTQVTVSS
TNF2-9GS-TNF2-9GS-ALB1(TNF25)	91	QVQLVESGGGLVQAGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKEREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLLMNSLK **PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSS**GGGGSGGGS EVQLVESGGGLVQAGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKEREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLLMNSLK PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSSGGGGSGGGS EVQLVESGGGLVQPGNSLRLSCAASGFTFRSFGMSWVRQAPGKEPE WVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTA VYYCTIGGSLSRSSQGTQVTVSS
TNF3-9GS-ALB1-9GS-TNF3(TNF26)	92	EVQLVESGGGLVQAGGSLSLSCSASGRSLSNYYMGWFRQAPGKERE LLGNISWRGYNIYYKDSVKGRFTISRDDAKNTIYLQMNRLKPEDTA VYYCAASILPLSDDPGWNTYWGQGTQVTVSSGGGGSGGGSEVQLVE SGGGLVQPGNSLRLSCAASGFTFRSFGMSWVRQAPGKEPEWVSSIS GSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAVYYCTI GGSLSRSSQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQAGGSLSL SCSASGRSLSNYYMGWFRQAPGKERELLGNISWRGYNIYYKDSVKG RFTISRDDAKNTIYLQMNRLKPEDTAVYYCAASILPLSDDPGWNTY WGQGTQVTVSS
TNF1-30GS-TNF1-9GS-ALB1(TNF27)	93	QVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTA LYYCARSPSGFNRGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGG GSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQ APGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNS LKPEDTALYYCARSPSGFNRGQGTQVTVSSGGGGSGGGSEVQLVES GGGLVQPGNSLRLSCAASGFTFRSFGMSWVRQAPGKEPEWVSSISG SGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAVYYCTIG GSLSRSSQGTQVTVSS
TNF3-30GS-TNF3-9GS-ALB1(TNF28)	94	EVQLVESGGGLVQAGGSLSLSCSASGRSLSNYYMGWFRQAPGKERE LLGNISWRGYNIYYKDSVKGRFTISRDDAKNTIYLQMNRLKPEDTA VYYCAASILPLSDDPGWNTYWGQGTQVTVSSGGGGSGGGGSGGGGS GGGGSGGGSSGGGGSEVQVVESGGGLVQAGGSLSLSCSASGRSLSN YYMGWFRQAPGKERELLGNISWRGYNIYYKDSVKGRFTISRDDAKN **TIYLQMNRLKPEDTAVYYCAASILPLSDDPGWNTYWGQGTQVTVSS** GGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFRSFGMSWV RQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQM NSLKPEDTAVYYCTIGGSLSRSSQGTQVTVSS
TNF30-9GS-ALB8-9GS-TNF30 (TNF60)	417	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTA VYYCARSPSGFNRGQGTLVTVSSggggsgggsEVQLVESGGGLVQP GNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLY ADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSS QGTLVTVSSggggsgggsEVQLVESGGGLVQPGGSLRLSCAASGFT FSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDN AKNTLYLQMNSLRPEDTAVYYCARSPSGFNRGQGTLVTVSS

TNF33-9GS-ALB8-9GS-TNF33 (TNF62)	418	EVQLVESGGGLVQPGGSLRLSCAASGRSLSNYYMGWFRQAPGKGRE LLGNISWRGYNIYYKDSVKGRFTISRDDSKNTIYLQMNSLRPEDTA VYYCAASILPLSDDPGWNTYWGQGTLVTVSSggggsgggsEVQLVE SGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSIS GSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTI GGSLSRSSQGTLVTVSSggggsgggsEVQLVESGGGLVQPGGSLRL SCAASGRSLSNYYMGWFRQAPGKGRELLGNISWRGYNIYYKDSVKG RFTISRDDSKNTIYLQMNSLRPEDTAVYYCAASILPLSDDPGWNTY WGQGTLVTVSS

Table 30

ID	Format
TNF24	TNF1-9GS-ALB1-9GS-TNF1
TNF25	TNF2-9GS-TNF2-9GS-ALB1
TNF26	TNF3-9GS-ALB1-9GS-TNF3
TNF27	TNF1-30GS-TNF1-9GS-ALB1
TNF28	TNF3-30GS-TNF3-9GS-ALB1

Table 31

Nanobody	Induction time	Yield (mg/L)
TNF24	ON/28°C	1.7
TNF25	short/37°C	0.445
TNF26	short/37°C	0.167
TNF27	ON/28°C	2.2
TNF28	short/37°C	1

Table 32

assay : ***L929s + Act D (5000c/w)***

TNF : ***human TNFa @ 0.5ng/ml***

	EC_{50} in nM			relative potency	
Nanobody	**mean**	**stdev**	**#**	**mean**	**stdev**
TNF24	0.011	0.003	11	0.878	0.248
TNF25	0.018	0.008	14	0.603	0.243
TNF26	0.020	0.009	14	0.583	0.210
TNF27	0.012	0.003	3	0.810	0.037
TNF28	0.021	0.008	6	0.548	0.360
Enbrel	0.009	0.005	45	1.002	0.011
Humira	0.079	0.043	39	0.097	0.069
Remicade	0.083	0.037	45	0.103	0.058

Table 33

Human albumin	KD (nM)	ka (1/Ms)	kd (1/s)
6A6 (ALB1)	0,57	1,11E+6	6,30E-4
1C2-GS-6A6-GS-1C2 (TNF24)	11	2,26E+05	2,48E-03
1G5-GS-1G5-GS-6A6 (TNF25)	7,2	2,91E+05	2,10E-03
5F10-GS-6A6-GS-5F10 (TNF26)	7,3	2,81E+05	2,05E-03
1C2-GS6-1C2-GS-6A6 (TNF27)	8,9	3,19E+05	2,84E-03
5F10-GS6-5F10-GS-6A6 (TNF28)	14	1,55E+05	2,13E-03

Table 34

%	untreated	RT	37C	50C	60C	70C	80C	90C
TNF24	100	100	99	98	5	3	8	18
TNF25	100	nd	103	102	95	5	4	6
TNF26	100	109	115	112	107	10	8	10
TNF27	100	102	103	102	22	9	26	34
TNF28	100	97	99	99	66	3	6	10

Table 35

Name	SEQ ID NO	Sequence
TNF29(TNF1 HUM1)	95	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTA VYYCARSPSGFNRGQGTLVTVSS
TNF30(TNF1 HUM2)	96	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTA VYYCARSPSGFNRGQGTLVTVSS
TNF31(TNF2 HUM1)	97	EVQLVESGGGLVQPGGSLRLSCAASGFTFSEPSGYTYTIGWFRQAP GKGREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLQMNSLR PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSS
TNF32(TNF2 HUM2)	98	EVQLVESGGGLVQPGGSLRLSCAASGFTFSEPSGYTYTIGWFRQAP GKGREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLQMNSLR PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTLVTVSS
TNF33(TNF3 HUM1)	99	EVQLVESGGGLVQPGGSLRLSCAASGRSLSNYYMGWFRQAPGKGRE LLGNISWRGYNIYYKDSVKGRFTISRDDSKNTIYLQMNSLRPEDTA VYYCAASILPLSDDPGWNTYWGQGTLVTVSS
ALB6(ALB1 HUM1)	100	EVQLVESGGGLVQPGNSLRLSCAASGFTFRSFGMSWVRQAPGKGLE WVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTA VYYCTIGGSLSRSSQGTLVTVSS
ALB7(ALB1 HUM2)	101	EVQLVESGGGLVQPGNSLRLSCAASGFTFRSFGMSWVRQAPGKGLE WVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTA VYYCTIGGSLSRSSQGTLVTVSS
ALB8(ALB1 HUM3)	102	EVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLE WVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTA VYYCTIGGSLSRSSQGTLVTVSS
ALB9(ALB1 HUM4)	103	EVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLE WVSSISGSGSDTLYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTA VYYCTIGGSLSRSSQGTLVTVSS
ALB10(ALB1 HUM5)	104	EVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLE WVSSISGSGSDTLYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTA VYYCTIGGSLSRSGQGTLVTVSS

Table 36

Nanobody™	Induction time	yield
TNF29	ON/28C	2.1 mg/L
TNF30	ON/28C	2.7 mg/L
TNF31	ON/28C	2 mg/L
TNF32	ON/28C	1.5 mg/L
TNF33	ON/28C	0.5 mg/L

289

Table 37

assay : ***L929s + Act D (5000c/w)***
TNF : ***human TNFa @ 0.5ng/ml***

	EC_{50} in nM			relative potency	
V_{HH}	mean	stdev	#	mean	stdev
TNF1	**0.707**	**0.265**	**14**	**0.015**	**0.007**
TNF13	0.988	0.014	3	0.014	0.003
TNF14	0.981	0.007	3	0.014	0.003
TNF29	1.336		1	0.013	
TNF30	0.985		1	0.017	
TNF2	**1.412**	**0.622**	**14**	**0.007**	**0.002**
TNF15	5.896	1.253	4	0.002	0.000
TNF16	2.422	0.192	4	0.005	0.001
TNF17	7.555	0.562	4	0.001	0.001
TNF18	3.134	0.481	4	0.004	0.001
TNF19	7.372	0.941	4	0.001	0.001
TNF31	2.195		1	0.008	
TNF32	2.506		1	0.007	
TNF3	**0.224**	**0.133**	**14**	**0.048**	**0.019**
TNF20	0.380	0.080	3	0.035	0.005
TNF21	0.889	0.019	3	0.015	0.003
TNF22	0.303	0.005	3	0.004	0.011
TNF23	0.3	0.011	3	0.04	0.011
TNF33	0.3		1	0.057	
Enbrel	0.009	0.005	45	1.002	0.011
Humira	0.079	0.043	39	0.097	0.069
Remicade	0.083	0.037	45	0.103	0.058

Table 38

	untreated	RT	37C	50C	60C	70C	80C	90C
TNF29	100	100	100	100	100	96	91	89
TNF30	100	100	100	99	100	96	92	89
TNF31	100	100	100	98	91	84	56	43
TNF32	100	99	98	97	87	78	45	39
TNF33	100	98	97	97	94	91	79	49

Table 39

assay : ***alphaKYM (10000c/w)***
TNF : ***human TNFa @ 1ng/ml***

Nanobody	EC_{50} in nM
TNF1	2.466
TNF2	4.236
TNF3	**0.655**
TNF4	0.069
TNF5	0.008
TNF6	0.121
TNF7	0.009
TNF8	0.013
TNF9	0.020
Enbrel	0.040
Humira	0.103
Remicade	**0.100**

Results from WO 04/41862

Nanobody	SEQ ID No	EC_{50} in nM
1A	1	100
3E	4	**12**
3G	5	20
Remicade		**0,080**

Table 40

M13_rev	SEQ ID NO: 421	GGATAACAATTTCACACAGG
Rev_9GlySer_L108	SEQ ID NO: 422	TCAGTAACCTGGATCCGCCACCGCTGCCTCCACCGCCTGAGGAGACGGTGACCAG
For_GS/Short	SEQ ID NO: 423	AGGTTACTGA**GGATCC**GAGGTGCAGCTGGTGGAGTCTGG
Rev_15BspEI_L108	SEQ ID NO: 424	TCAGTAACCTTCCGGAACCGCCACCGCCTGAGGAGACGGTGACAAG
For_BspEI	SEQ ID NO: 425	AGGTTACTGA**TCCGGA**GGCGGTAGCGAGGTGCAGCTGGTGGAGTCTGG
M13_for	SEQ ID NO: 426	CACGACGTTGTAAAACGAC

Table 41

Reverse primer		**Sequence**
PiRevhumNot/a40c (Not1)	SEQ ID NO: 427	ATGGTGGTGT**GCGGCCGC**CTATTATGAGGAGACGGTGACCAGG
Forward primer		
Pi2for (Xho1)	SEQ ID NO: 428	AGGGGTATCT**CTCGAG**AAAAGAGAGGTGCAGCTGGTGGAGTCTGG

Table 42

Human TNFα	**EC_{50} in nM**		
VHH	**mean**	**stdev**	**Number of assays**
TNF60	0.010	0.002	6
Enbrel	0.014	0.009	33
Humira	0.141	0.074	33
Remicade	0.120	0.037	33

Table 43

		human albumin	rhesus albumin
TNF60	K_D (nM)	**24,4**	**24,1**
	k_{on} (1/Ms)	2,05E+05	2,09E+05
	k_{off} (1/s)	5,02E-03	5,04E-03
TNF24	K_D (nM)	**11**	Nd
	k_{on} (1/Ms)	2.26E+05	Nd
	k_{off} (1/s)	2.48E-03	Nd

292

Table 44

PiForLong	SEQ ID NO: 429	GCTAAAGAAGAAGGGGTATCTCTCGAGAAAAGAGAGGTGCAGCTGGTGGAGTCTGG
Rev_30GlySer_L108	SEQ ID NO: 430	TCAGTAACCTGGATCCCCCGCCACCGCTGCCTCCACCGCCGCTACCCCCGCCACCGC TGCCTCCACCGCCTGAGGAGACGGTGACAAG
For_GlySer	SEQ ID NO: 431	AGGTTACTGA**GGATCC**GGCGGTGGAGGCAGCGGTGGCGGGGGTAGCGAGGTGCAGCTGGTGGAGTCTGG
PiRevCys1hum	SEQ ID NO: 432	ATGGTGGTGTGAATTCTTATTAGCAGGAGACGGTGACAAGG
PiRevCys2hum	SEQ ID NO: 433	ATGGTGGTGTGAATTCTTATTAGCAACCTCCACCTGAGGAGACGGTGACAAGG
AOXIFor	SEQ ID NO: 434	GACTGGTTCCAATTGACAAGC
AOXIRev	SEQ ID NO: 435	GCAAATGGCATTCTGACATCC

Table 45

Human TNFα	EC_{50} in nM		
VHH	**mean**	**stdev**	**Number of assays**
TNF1	0.748	0.153	27
TNF55-PEG40	0.004	0.001	8
TNF55-PEG60	0.004	0.002	6
TNF55-Biotine	0.012	0.003	5
TNF56-PEG40	0.006	0.003	57
TNF56-PEG60	0.005	0.003	7
TNF56-Biotine	0.017	0.009	13
Enbrel	0.013	0.006	71
Humira	0.127	0.058	67
Remicade	0.144	0.061	67

5

Table 46

	DS534 P4	DS592 P4	DS605 P3	KM05-179 P4
p[IC]50				
Etanercept	9.55	9.49	9.51	9.51
Accipiter	9.88	9.44	9.37	9.27
pM				
Etanercept	282	324	309	309
Accipiter	132	363	427	537

Table 47

	Total WBC count ($x10^6$/mL)	
Dose Group	ALX0071	Etanercept
CMC vehicle	0.86 ± 0.09	
0.1 μg human TNFα	3.72 ± 0.21	
0.0625 mg/kg	3.03 ± 0.6	-
0.125 mg/kg	1.23 ± 0.3**	2.40 ± 0.39
0.25 mg/kg	1.37 ± 0.17*	2.47 ± 0.54
0.5 mg/kg	-	2.19 ± 0.10

*P<0.05, **P<0.01 vs 0.1 μg human TNFα

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

All references disclosed herein are incorporated by reference in their entirety.

SEQUENCE LISTING

<110> Ablynx N.V.

<120> Improved Nanobodies™ against Tumor Necrosis Factor-alpha

<130> P05-004PCT

<160> 435

<170> PatentIn version 3.1

<210> 1

<211> 26

<212> PRT

<213> -

<220>

<221> misc_feature

<222> (11)..(11)

<223> Hallmark residue

<400> 1

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Xaa Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
20 25

<210> 2

<211> 14

<212> PRT

<213> -

<220>

<221> misc_feature

<222> (2)..(2)

<223> Hallmark residue

<220>

<221> misc_feature

<222> (9)..(9)

<223> Hallmark residue

<220>

<221> misc_feature

<222> (10)..(10)

<223> Hallmark residue

<220>

<221> misc_feature

<222> (12)..(12)

<223> Hallmark residue

<400> 2

Trp Xaa Arg Gln Ala Pro Gly Lys Xaa Xaa Glu Xaa Val Ala
1 5 10

<210> 3

<211> 32

<212> PRT

<213> -

<220>

<221> misc_feature

<222> (21)..(21)

<223> Hallmark residue

<220>

<221> misc_feature

<222> (22)..(22)

<223> Hallmark residue

<400> 3

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Xaa Xaa Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 4

<211> 11

<212> PRT

<213> -

<220>

<221> misc_feature

<222> (1)..(1)

<223> Hallmark residue

<220>

<221> misc_feature

<222> (2)..(2)

<223> Hallmark residue

<220>

<221> misc_feature

<222> (6)..(6)

<223> Hallmark residue

<400> 4

Xaa Xaa Gln Gly Thr Xaa Val Thr Val Ser Ser
1 5 10

<210> 5

<211> 26

<212> PRT

<213> -

<400> 5

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
20 25

<210> 6

<211> 14

<212> PRT

<213> -

<400> 6

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Val Ala
1 5 10

<210> 7

<211> 14

<212> PRT

<213> -

<400> 7

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 8

<211> 13

<212> PRT

<213> -

<400> 8

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Ala
1 5 10

<210> 9

<211> 14

<212> PRT

<213> -

<400> 9

Trp Phe Arg Gln Ala Pro Gly Lys Gln Arg Glu Leu Val Ala
1 5 10

<210> 10

<211> 14

<212> PRT

<213> -

<400> 10

Trp Phe Arg Gln Ala Pro Gly Lys Gln Arg Glu Phe Val Ala
1 5 10

<210> 11

<211> 13

<212> PRT

<213> -

<400> 11

Trp Tyr Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ala
1 5 10

<210> 12

<211> 32

<212> PRT

<213> -

<400> 12

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 13

<211> 11

<212> PRT

<213> -

<400> 13

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 14

<211> 11

<212> PRT

<213> -

<400> 14

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> 15

<211> 5

<212> PRT

<213> -

<400> 15

Asp Tyr Trp Met Tyr
1 5

<210> 16

<211> 10

<212> PRT

<213> -

<400> 16

Glu Pro Ser Gly Tyr Thr Tyr Thr Ile Gly
1 5 10

<210> 17

<211> 5

<212> PRT

<213> -

<400> 17

Asn Tyr Tyr Met Gly
1 5

<210> 18

<211> 5

<212> PRT

<213> -

<400> 18

Val Ser Trp Met Tyr
1 5

<210> 19

<211> 8

<212> PRT

<213> -

<400> 19

Ala His Ser Val Tyr Thr Met Gly
1 5

<210> 20

<211> 5

<212> PRT

<213> -

<400> 20

Gly Tyr Tyr Met Gly
1 5

<210> 21

<211> 10

<212> PRT

<213> -

<400> 21

Asp Tyr Ser Gly Tyr Thr Tyr Thr Val Gly
1 5 10

<210> 22

<211> 17

<212> PRT

<213> -

<400> 22

Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val Lys
1 5 10 15

Gly

<210> 23

<211> 17

<212> PRT

<213> -

<400> 23

Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 24

<211> 17

<212> PRT

<213> -

<400> 24

Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val Lys
1 5 10 15

Gly

<210> 25

<211> 17

<212> PRT

<213> -

<400> 25

Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Val Asp Ser Val Lys
1 5 10 15

Gly

<210> 26

<211> 17

<212> PRT

<213> -

<400> 26

Arg Ile Tyr Trp Ser Ser Gly Asn Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 27

<211> 17

<212> PRT

<213> -

<400> 27

Arg Ile Tyr Trp Ser Ser Ala Asn Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 28

<211> 17

<212> PRT

<213> -

<400> 28

Ser Ile Ser Trp Arg Gly Asp Asn Thr Tyr Tyr Lys Glu Ser Val Lys
1 5 10 15

Gly

<210> 29

<211> 15

<212> PRT

<213> -

<400> 29

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Gly Ser Tyr Asn Tyr
1 5 10 15

<210> 30

<211> 14

<212> PRT

<213> -

<400> 30

Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
1 5 10

<210> 31

<211> 15

<212> PRT

<213> -

<400> 31

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Glu Ala Tyr Asn Tyr
1 5 10 15

<210> 32

<211> 14

<212> PRT

<213> -

<400> 32

Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Asn
1 5 10

<210> 33

<211> 15

<212> PRT

<213> -

<400> 33

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Glu Ser Tyr Asn Tyr
1 5 10 15

<210> 34

<211> 6

<212> PRT

<213> -

<400> 34

Ser Pro Ser Gly Phe Asn
1 5

<210> 35

<211> 6

<212> PRT

<213> -

<400> 35

Ser Pro Ser Gly Ser Phe
1 5

<210> 36

<211> 5

<212> PRT

<213> -

<400> 36

Ser Phe Gly Met Ser
1 5

<210> 37

<211> 5

<212> PRT

<213> -

<400> 37

Leu Asn Leu Met Gly
1 5

<210> 38

<211> 5

<212> PRT

<213> -

<400> 38

Ile Asn Leu Leu Gly
1 5

<210> 39

<211> 5

<212> PRT

<213> -

<400> 39

Asn Tyr Trp Met Tyr
1 5

<210> 40

<211> 17

<212> PRT

<213> -

<400> 40

Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 41

<211> 16

<212> PRT

<213> -

<400> 41

Thr Ile Thr Val Gly Asp Ser Thr Asn Tyr Ala Asp Ser Val Lys Gly
1 5 10 15

<210> 42

<211> 16

<212> PRT

<213> -

<400> 42

Thr Ile Thr Val Gly Asp Ser Thr Ser Tyr Ala Asp Ser Val Lys Gly
1 5 10 15

<210> 43

<211> 17

<212> PRT

<213> -

<400> 43

Ser Ile Asn Gly Arg Gly Asp Asp Thr Arg Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 44

<211> 17

<212> PRT

<213> -

<400> 44

Ala Ile Ser Ala Asp Ser Ser Thr Lys Asn Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 45

<211> 17

<212> PRT

<213> -

<400> 45

Ala Ile Ser Ala Asp Ser Ser Asp Lys Arg Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 46

<211> 17

<212> PRT

<213> -

<400> 46

Arg Ile Ser Thr Gly Gly Gly Tyr Ser Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 47

<211> 13

<212> PRT

<213> -

<400> 47

Asp Arg Glu Ala Gln Val Asp Thr Leu Asp Phe Asp Tyr
1 5 10

<210> 48

<211> 6

<212> PRT

<213> -

<400> 48

Gly Gly Ser Leu Ser Arg
1 5

<210> 49

<211> 8

<212> PRT

<213> -

<400> 49

Arg Arg Thr Trp His Ser Glu Leu
1 5

<210> 50

<211> 7

<212> PRT

<213> -

<400> 50

Gly Arg Ser Val Ser Arg Ser
1 5

<210> 51

<211> 5

<212> PRT

<213> -

<400> 51

Gly Arg Gly Ser Pro
1 5

<210> 52

<211> 115

<212> PRT

<213> -

<400> 52

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 53

<211> 115

<212> PRT

<213> -

<400> 53

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Met Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Gly Val Ser
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Ser Phe Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 54

<211> 115

<212> PRT

<213> -

<400> 54

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Asp Phe Ser Val Ser
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Val Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asp Ser Leu Ile Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Ser Phe Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 55

<211> 129

<212> PRT

<213> -

<400> 55

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Glu Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Leu Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 56

<211> 129

<212> PRT

<213> -

<400> 56

Gln Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Asp Tyr
20 25 30

Ser Gly Tyr Thr Tyr Thr Val Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Glu Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Asn Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Leu Met Asn Asn Leu Glu Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Glu Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 57

<211> 129

<212> PRT

<213> -

<400> 57

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asp

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Leu Met Asn Asn Leu Glu Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Glu Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 58

<211> 127

<212> PRT

<213> -

<400> 58

Ala Val Gln Leu Val Asp Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Ala His
20 25 30

Ser Val Tyr Thr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg
35 40 45

Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Ala Asn Thr Tyr Tyr Ala
50 55 60

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
65 70 75 80

Thr Val Asp Leu Leu Met Asn Cys Leu Lys Pro Glu Asp Thr Ala Val
85 90 95

Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Glu
100 105 110

Ala Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120 125

<210> 59

<211> 123

<212> PRT

<213> -

<400> 59

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ala Ala Ser Gly Arg Ser Phe Thr Gly Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Gln Leu Leu
35 40 45

Ala Ser Ile Ser Trp Arg Gly Asp Asn Thr Tyr Tyr Lys Glu Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Asn
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 60

<211> 123

<212> PRT

<213> -

<400> 60

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 61

<211> 117

<212> PRT

<213> -

<400> 61

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Gly Gly Gly
1 5 10 15

Ser Leu Arg Leu Ala Cys Ala Ala Ser Glu Arg Ile Phe Asp Leu Asn
20 25 30

Leu Met Gly Trp Tyr Arg Gln Gly Pro Gly Asn Glu Arg Glu Leu Val
35 40 45

Ala Thr Cys Ile Thr Val Gly Asp Ser Thr Asn Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Met Asp Tyr Thr Lys Gln Thr Val Tyr
65 70 75 80

Leu His Met Asn Ser Leu Arg Pro Glu Asp Thr Gly Leu Tyr Tyr Cys
85 90 95

Lys Ile Arg Arg Thr Trp His Ser Glu Leu Trp Gly Gln Gly Thr Gln
100 105 110

Val Thr Val Ser Ser
115

<210> 62

<211> 116

<212> PRT

<213> -

<400> 62

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Glu Gly Gly
1 5 10 15

Ser Leu Arg Leu Ala Cys Ala Ala Ser Glu Arg Ile Trp Asp Ile Asn
20 25 30

Leu Leu Gly Trp Tyr Arg Gln Gly Pro Gly Asn Glu Arg Glu Leu Val
35 40 45

Ala Thr Ile Thr Val Gly Asp Ser Thr Ser Tyr Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Tyr Asp Lys Asn Thr Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Gly Leu Tyr Tyr Cys Lys
85 90 95

Ile Arg Arg Thr Trp His Ser Glu Leu Trp Gly Gln Gly Thr Gln Val
100 105 110

Thr Val Ser Ser
115

<210> 63

<211> 115

<212> PRT

<213> -

<400> 63

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 64

<211> 116

<212> PRT

<213> -

<400> 64

Ala Val Gln Leu Val Asp Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Phe Gly Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Tyr Pro Gly Lys Glu Pro Glu Trp Val
35 40 45

Ser Ser Ile Asn Gly Arg Gly Asp Asp Thr Arg Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Ser Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Glu Tyr Tyr Cys
85 90 95

Thr Ile Gly Arg Ser Val Ser Arg Ser Arg Thr Gln Gly Thr Gln Val
100 105 110

Thr Val Ser Ser
115

<210> 65

<211> 114

<212> PRT

<213> -

<400> 65

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Thr Cys Thr Ala Ser Gly Phe Thr Phe Arg Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Asp Gln Glu Trp Val
35 40 45

Ser Ala Ile Ser Ala Asp Ser Ser Thr Lys Asn Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Lys Met Leu Tyr
65 70 75 80

Leu Glu Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Val Ile Gly Arg Gly Ser Pro Ser Ser Pro Gly Thr Gln Val Thr Val
100 105 110

Ser Ser

<210> 66

<211> 114

<212> PRT

<213> -

<400> 66

Gln Val Gln Leu Ala Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Thr Cys Thr Ala Ser Gly Phe Thr Phe Gly Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Glu Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Ala Asp Ser Ser Asp Lys Arg Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Lys Met Leu Tyr
65 70 75 80

Leu Glu Met Asn Ser Leu Lys Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Val Ile Gly Arg Gly Ser Pro Ala Ser Gln Gly Thr Gln Val Thr Val
100 105 110

Ser Ser

<210> 67

<211> 123

<212> PRT

<213> -

<400> 67

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30

Trp Met Tyr Trp Val Arg Val Ala Pro Gly Lys Gly Leu Glu Arg Ile
35 40 45

Ser Arg Asp Ile Ser Thr Gly Gly Gly Tyr Ser Tyr Tyr Ala Asp Ser
50 55 60

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu
65 70 75 80

Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr
85 90 95

Cys Ala Lys Asp Arg Glu Ala Gln Val Asp Thr Leu Asp Phe Asp Tyr
100 105 110

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 68

<211> 9

<212> PRT

<213> -

<400> 68

Gly Gly Gly Gly Ser Gly Gly Gly Ser
1 5

<210> 69

<211> 30

<212> PRT

<213> -

<400> 69

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
20 25 30

<210> 70

<211> 239

<212> PRT

<213> -

<400> 70

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Gln Val Gln Leu
115 120 125

Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu
130 135 140

Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr Trp Met Tyr Trp
145 150 155 160

Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Glu Ile Asn
165 170 175

Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val Lys Gly Arg Phe
180 185 190

Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met Asn
195 200 205

Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg Ser Pro
210 215 220

Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
225 230 235

<210> 71

<211> 267

<212> PRT

<213> -

<400> 71

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Glu Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Leu Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Gln Val Gln Leu Val Glu
130 135 140

Ser Gly Gly Gly Leu Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys
145 150 155 160

Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro Ser Gly Tyr Thr Tyr Thr
165 170 175

Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
180 185 190

Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr Tyr Ala Asp Ser Val Lys
195 200 205

Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala Lys Asn Thr Val Asp Leu
210 215 220

Leu Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala
225 230 235 240

Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Gly Ser Tyr Asn Tyr
245 250 255

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
260 265

<210> 72

<211> 255

<212> PRT

<213> -

<400> 72

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val
130 135 140

Gln Ala Gly Gly Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser
145 150 155 160

Leu Ser Asn Tyr Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu
165 170 175

Arg Glu Leu Leu Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr
180 185 190

Lys Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys
195 200 205

Asn Thr Ile Tyr Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala
210 215 220

Val Tyr Tyr Cys Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly
225 230 235 240

Trp Asn Thr Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
245 250 255

<210> 73

<211> 260

<212> PRT

<213> -

<400> 73

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
145 150 155 160

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp
165 170 175

Tyr Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
180 185 190

Val Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser
195 200 205

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu
210 215 220

Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr
225 230 235 240

Cys Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val
245 250 255

Thr Val Ser Ser
260

<210> 74

<211> 288

<212> PRT

<213> -

<400> 74

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Glu Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Leu Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
130 135 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln
145 150 155 160

Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly Ser
165 170 175

Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro Ser
180 185 190

Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu
195 200 205

Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr Tyr
210 215 220

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala Lys
225 230 235 240

Asn Thr Val Asp Leu Leu Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
245 250 255

Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser Val
260 265 270

Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
275 280 285

<210> 75

<211> 276

<212> PRT

<213> -

<400> 75

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
130 135 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser
145 150 155 160

Gly Gly Gly Leu Val Gln Ala Gly Gly Ser Leu Ser Leu Ser Cys Ser
165 170 175

Ala Ser Gly Arg Ser Leu Ser Asn Tyr Tyr Met Gly Trp Phe Arg Gln
180 185 190

Ala Pro Gly Lys Glu Arg Glu Leu Leu Gly Asn Ile Ser Trp Arg Gly
195 200 205

Tyr Asn Ile Tyr Tyr Lys Asp Ser Val Lys Gly Arg Phe Thr Ile Ser
210 215 220

Arg Asp Asp Ala Lys Asn Thr Ile Tyr Leu Gln Met Asn Arg Leu Lys
225 230 235 240

Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala Ser Ile Leu Pro Leu
245 250 255

Ser Asp Asp Pro Gly Trp Asn Thr Tyr Trp Gly Gln Gly Thr Gln Val
260 265 270

Thr Val Ser Ser
275

<210> 76

<211> 115

<212> PRT

<213> -

<400> 76

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 77

<211> 115

<212> PRT

<213> -

<400> 77

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 78

<211> 129

<212> PRT

<213> -

<400> 78

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 79

<211> 129

<212> PRT

<213> -

<400> 79

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 80

<211> 129

<212> PRT

<213> -

<400> 80

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 81

<211> 129

<212> PRT

<213> -

<400> 81

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 82

<211> 129

<212> PRT

<213> -

<400> 82

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
115 120 125

Ser

<210> 83

<211> 123

<212> PRT

<213> -

<400> 83

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 84

<211> 123

<212> PRT

<213> -

<400> 84

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 85

<211> 123

<212> PRT

<213> -

<400> 85

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 86

<211> 123

<212> PRT

<213> -

<400> 86

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 87

<211> 115

<212> PRT

<213> -

<400> 87

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 88

<211> 115

<212> PRT

<213> -

<400> 88

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 89

<211> 115

<212> PRT

<213> -

<400> 89

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 90

<211> 363

<212> PRT

<213> -

<400> 90

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
            20                  25                  30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr
            100                 105                 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln Leu
        115                 120                 125

Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn Ser Leu Arg Leu
    130                 135                 140

Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe Gly Met Ser Trp
145                 150                 155                 160

Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val Ser Ser Ile Ser
                165                 170                 175

Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val Lys Gly Arg Phe
            180                 185                 190

Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr Leu Gln Met Asn
        195                 200                 205

Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Thr Ile Gly Gly
    210                 215                 220

Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr Val Ser Ser Gly
225                 230                 235                 240
```

Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly
245 250 255

Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala
260 265 270

Ser Gly Phe Thr Phe Ser Asp Tyr Trp Met Tyr Trp Val Arg Gln Ala
275 280 285

Pro Gly Lys Gly Leu Glu Trp Val Ser Glu Ile Asn Thr Asn Gly Leu
290 295 300

Ile Thr Lys Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg
305 310 315 320

Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys Pro
325 330 335

Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg Ser Pro Ser Gly Phe Asn
340 345 350

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
355 360

<210> 91

<211> 391

<212> PRT

<213> -

<400> 91

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Glu Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Leu Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln Leu Val Glu
130 135 140

Ser Gly Gly Gly Leu Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys
145 150 155 160

Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro Ser Gly Tyr Thr Tyr Thr
165 170 175

Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
180 185 190

Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr Tyr Ala Asp Ser Val Lys
195 200 205

Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala Lys Asn Thr Val Asp Leu
210 215 220

Leu Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala
225 230 235 240

Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Gly Ser Tyr Asn Tyr
245 250 255

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser
260 265 270

Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val
275 280 285

Gln Pro Gly Asn Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
290 295 300

Phe Arg Ser Phe Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Glu
305 310 315 320

Pro Glu Trp Val Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr
325 330 335

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
340 345 350

Thr Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
355 360 365

Val Tyr Tyr Cys Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly
370 375 380

Thr Gln Val Thr Val Ser Ser
385 390

<210> 92

<211> 379

<212> PRT

<213> -

<400> 92

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val
130 135 140

Gln Pro Gly Asn Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
145 150 155 160

Phe Arg Ser Phe Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Glu
165 170 175

Pro Glu Trp Val Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr
180 185 190

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
195 200 205

Thr Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
210 215 220

Val Tyr Tyr Cys Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly
225 230 235 240

Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser
245 250 255

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
260 265 270

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser Asn Tyr
275 280 285

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu
290 295 300

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
305 310 315 320

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
325 330 335

Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
340 345 350

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
355 360 365

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
370 375

<210> 93

<211> 384

<212> PRT

<213> -

<400> 93

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
145 150 155 160

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp
165 170 175

Tyr Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
180 185 190

Val Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser
195 200 205

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu
210 215 220

Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr
225 230 235 240

Cys Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val
245 250 255

Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln
260 265 270

Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn Ser Leu Arg
275 280 285

Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe Gly Met Ser
290 295 300

Trp Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val Ser Ser Ile
305 310 315 320

Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val Lys Gly Arg
325 330 335

Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr Leu Gln Met
340 345 350

Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Thr Ile Gly
355 360 365

Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr Val Ser Ser
370 375 380

<210> 94

<211> 400

<212> PRT

<213> -

<400> 94

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
130 135 140

Gly Gly Ser Ser Gly Gly Gly Gly Ser Glu Val Gln Val Val Glu Ser
145 150 155 160

Gly Gly Gly Leu Val Gln Ala Gly Gly Ser Leu Ser Leu Ser Cys Ser
165 170 175

Ala Ser Gly Arg Ser Leu Ser Asn Tyr Tyr Met Gly Trp Phe Arg Gln
180 185 190

Ala Pro Gly Lys Glu Arg Glu Leu Leu Gly Asn Ile Ser Trp Arg Gly
195 200 205

Tyr Asn Ile Tyr Tyr Lys Asp Ser Val Lys Gly Arg Phe Thr Ile Ser
210 215 220

Arg Asp Asp Ala Lys Asn Thr Ile Tyr Leu Gln Met Asn Arg Leu Lys
225 230 235 240

Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala Ser Ile Leu Pro Leu
245 250 255

Ser Asp Asp Pro Gly Trp Asn Thr Tyr Trp Gly Gln Gly Thr Gln Val
260 265 270

Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln
275 280 285

Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn Ser Leu Arg
290 295 300

Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe Gly Met Ser
305 310 315 320

Trp Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val Ser Ser Ile
325 330 335

Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val Lys Gly Arg
340 345 350

Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr Leu Gln Met
355 360 365

Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Thr Ile Gly
370 375 380

Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr Val Ser Ser
385 390 395 400

<210> 95

<211> 115

<212> PRT

<213> -

<400> 95

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 96

<211> 115

<212> PRT

<213> -

<400> 96

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 97

<211> 129

<212> PRT

<213> -

<400> 97

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 98

<211> 129

<212> PRT

<213> -

<400> 98

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
115 120 125

Ser

<210> 99

<211> 123

<212> PRT

<213> -

<400> 99

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 100

<211> 115

<212> PRT

<213> -

<400> 100

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 101

<211> 115

<212> PRT

<213> -

<400> 101

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 102

<211> 115

<212> PRT

<213> -

<400> 102

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 103

<211> 115

<212> PRT

<213> -

<400> 103

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 104

<211> 115

<212> PRT

<213> -

<400> 104

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 105

<211> 130

<212> PRT

<213> -

<400> 105

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gln Ile Ile Phe Gly Ser His
20 25 30

Val Ala Ala Trp Phe Arg Gln Ala Pro Gly Arg Glu Arg Glu Phe Val
35 40 45

Ala Glu Ile Arg Pro Ser Gly Asp Phe Gly Pro Glu Gly Glu Phe Glu
50 55 60

His Val Thr Ala Ser Leu Lys Gly Arg Phe Thr Ile Ala Lys Asn Ser
65 70 75 80

Val Asp Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp
85 90 95

Thr Ala Val Tyr Tyr Cys Ala Ala Ala Pro Tyr Arg Gly Gly Arg Asp
100 105 110

Tyr Arg Trp Glu Tyr Glu Tyr Glu Tyr Trp Gly Gln Gly Thr Gln Val
115 120 125

Thr Val
130

<210> 106

<211> 122

<212> PRT

<213> -

<400> 106

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Asn Ala Gly Ser Thr Ser Asn Ala Tyr
20 25 30

Ala Thr Gly Trp Phe Arg Arg Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Gly Ile Gln Trp Ser Gly Gly Asp Ala Phe Tyr Arg Asn Ser Val
50 55 60

Lys Gly Arg Phe Arg Ile Thr Arg Asp Pro Asp Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Asp Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr Cys Ala
85 90 95

Gln Lys Leu Ser Pro Tyr Tyr Asn Asp Phe Asp Ser Ser Asn Tyr Glu
100 105 110

Tyr Trp Gly Gln Gly Thr Gln Val Thr Val
115 120

<210> 107

<211> 119

<212> PRT

<213> -

<400> 107

Glu Val Gln Leu Val Glu Ser Gly Gly Asp Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Gln Leu Phe Ser Thr Asn
20 25 30

Asp Val Gly Trp Tyr Arg Arg Ala Pro Gly Lys Gln Arg Glu Leu Val
35 40 45

Ala Thr Ile Thr Asp Asp Gly Thr Thr Asp Tyr Gly Asp Asp Val Lys
50 55 60

Gly Arg Phe Val Ile Ser Arg Glu Gly Glu Met Val Tyr Leu Glu Met
65 70 75 80

Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Asn Ile Asn
85 90 95

Arg Leu Arg Ser Thr Trp Gly Ile Arg Tyr Asp Val Trp Gly Gln Gly
100 105 110

Thr Gln Val Thr Val Ser Ser
115

<210> 108

<211> 115

<212> PRT

<213> -

<400> 108

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Val Ser Gly Phe Thr Phe Ser Thr Thr
20 25 30

Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Phe Glu Glu Trp Val
35 40 45

Ser Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Gly Arg Arg Gly Tyr Gly Arg Asp Arg Ser Lys Gly Ile Gln Val Thr
100 105 110

Val Ala Ser
115

<210> 109

<211> 121

<212> PRT

<213> -

<400> 109

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Thr Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Phe Ser Ser Val
20 25 30

Ala Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gln Arg Glu Phe Leu
35 40 45

Ala Gly Val Gly Tyr Asp Gly Ser Ser Ile Arg Tyr Ala Glu Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ala Arg Gly Asn Arg Glu Ser Thr Val Phe
65 70 75 80

Leu Gln Met Glu Asn Leu Lys Pro Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95

Thr Ala Glu Pro Ile Gly Ala Tyr Glu Gly Leu Trp Thr Tyr Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 110

<211> 124

<212> PRT

<213> -

<220>

<221> misc_feature

<222> (1)..(4)

<223> unknown amino acid residues, probably EVQL, QVQL or a similar seq uence

<400> 110

Xaa Xaa Xaa Xaa Val Glu Ser Gly Gly Gly Leu Met Gln Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Met Phe Ser Asp Ser
20 25 30

Ala Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Thr Ile Ser Trp Asn Gly Gly Ser Ser Ser Tyr Ala Asp Phe Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Gly Leu Thr Pro Gln Asp Thr Ala Ile Tyr Tyr Cys
85 90 95

Ala Gly Ser Tyr Ser Asn Gly Asn Pro His Arg Phe Ser Gln Tyr Gln
100 105 110

Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 111

<211> 120

<212> PRT

<213> -

<220>

<221> misc_feature

<222> (77)..(77)

<223> Unknown amino acid residue

<400> 111

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Gly Thr Tyr
20 25 30

Ala Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Ala Ile Ser Trp Gly Gly Gly Ser Ile Val Tyr Ala Glu Ser Ala
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Xaa Thr Met Tyr
65 70 75 80

Leu Gln Met Asp Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ala Asn Asn Ile Ala Thr Leu Arg Gln Gly Ser Trp Gly Gln
100 105 110

Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 112

<211> 127

<212> PRT

<213> -

<400> 112

Glu Val Gln Leu Val Glu Ser Gly Gly Glu Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Arg Asn Phe Val Thr Tyr
20 25 30

Ala Met Ser Trp Phe Arg Arg Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Ser Ile Ser Trp Ser Gly Asp Thr Thr Tyr Tyr Ser Asn Ser Val
50 55 60

Lys Gly Arg Phe Thr Val Ser Arg Asp Asn Gly Lys Asn Thr Ala Tyr
65 70 75 80

Leu Arg Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Asp Tyr Tyr Cys
85 90 95

Ala Val Val Gln Val Ile Asp Pro Ser Trp Ser Gly Val Asn Leu Asp
100 105 110

Asp Tyr Asp Tyr Leu Gly Ser Gly Thr Gln Val Thr Val Ser Ser
115 120 125

<210> 113

<211> 124

<212> PRT

<213> -

<400> 113

Glu Val Gln Leu Val Glu Ser Gly Gly Arg Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Asn Ala Gly Ser Thr Ser Asn Ala Tyr
20 25 30

Ala Thr Gly Trp Phe Arg Arg Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Gly Ile Gln Trp Ser Gly Gly Asp Ala Phe Tyr Arg Asn Ser Val
50 55 60

Lys Gly Arg Phe Arg Ile Thr Arg Asp Pro Asp Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Asp Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr Cys Ala
85 90 95

Gln Lys Leu Ser Pro Tyr Tyr Asn Asp Phe Asp Ser Ser Asn Tyr Glu
100 105 110

Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 114

<211> 115

<212> PRT

<213> -

<400> 114

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Ala Thr Ile Ser Ser Ile Val
20 25 30

Met Leu Gly Trp Tyr Arg Gln Ala Pro Gly Lys Gln Arg Glu Trp Val
35 40 45

Ala Ser Ile Thr Ile Gly Ser Arg Thr Asn Tyr Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Phe Cys Asn
85 90 95

Ala Val Pro Pro Arg Asp Asp Tyr Trp Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 115

<211> 121

<212> PRT

<213> -

<400> 115

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Gln Thr Ser Ser Ser Tyr
20 25 30

Asp Met Gly Trp Phe Arg Gln Ala Pro Gly Glu Gly Arg Glu Phe Val
35 40 45

Ala Arg Ile Ser Gly Ser Asp Gly Ser Thr Tyr Tyr Ser Asp Arg Ala
50 55 60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Thr Lys Asn Met Val Tyr
65 70 75 80

Leu Gln Met Asp Arg Leu Lys Pro Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Arg Val Pro Arg Tyr Glu Asn Gln Trp Ser Ser Tyr Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 116

<211> 127

<212> PRT

<213> -

<400> 116

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ser Thr Phe Ser Thr Tyr
20 25 30

Asp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Asp Ser Gly Gly Gly Ser Pro Met Tyr Val Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Val Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Phe Ser Thr Gly Ala Asp Gly Gly Ser Trp Tyr Trp Ser Tyr
100 105 110

Gly Met Asp Ser Trp Gly Lys Gly Thr Gln Val Thr Val Ser Ser
115 120 125

<210> 117

<211> 121

<212> PRT

<213> -

<400> 117

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Glu Ala Ser Glu Arg Ser Ser Asn Arg Tyr
20 25 30

Asn Met Ala Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Leu
35 40 45

Ala Arg Val Asp Val Ser Gly Gly Asn Thr Leu Tyr Gly Asp Ser Val
50 55 60

Lys Asp Arg Phe Thr Val Ser Arg Ile Asn Gly Lys Asn Ala Met Tyr
65 70 75 80

Leu Gln Met Asn Asn Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr Cys
85 90 95

Ala Ala Gly Gly Trp Gly Thr Thr Gln Tyr Asp Tyr Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 118

<211> 117

<212> PRT

<213> -

<400> 118

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Cys Val Ser Ser Gly Cys Thr Phe Ser
20 25 30

Ala Tyr Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Ala Glu Glu
35 40 45

Trp Val Ser Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp
50 55 60

Ser Val Asn Gly Arg Phe Lys Ile Ser Arg Asp Asn Ala Lys Lys Thr
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Gly Pro Glu Asp Thr Ala Met Tyr
85 90 95

Tyr Cys Gln Arg Arg Gly Tyr Ala Leu Asp Arg Gly Gln Gly Thr Gln
100 105 110

Val Thr Val Ser Ser
115

<210> 119

<211> 129

<212> PRT

<213> -

<400> 119

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Thr Leu Ser Cys Ala Ser Ser Gly Arg Gly Phe Tyr Lys Asn
20 25 30

Ala Met Gly Trp Phe Arg Gln Pro Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Ser Ile Lys Trp Asn Gly Asn Asn Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Arg Gly Arg Phe Thr Ile Ser Arg Gly Asn Ala Lys Asn Thr Glu Asn
65 70 75 80

Thr Val Ser Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Asp
85 90 95

Tyr Tyr Cys Ala Ala Asp Ser Ser His Tyr Ser Tyr Val Tyr Ser Lys
100 105 110

Ala Tyr Glu Tyr Asp Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 120

<211> 115

<212> PRT

<213> -

<400> 120

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Phe Ser Gly Phe Ala Phe Ser Ala Ser
20 25 30

Ser Met Ala Trp Val Arg Gln Ala Pro Gly Lys Tyr Glu Glu Trp Val
35 40 45

Ser Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val
50 55 60

Gln Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Gly Arg Arg Gly Tyr Gly Arg Asp Arg Ser Gln Gly Ile Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 121

<211> 127

<212> PRT

<213> -

<220>

<221> misc_feature

<222> (119)..(119)

<223> Unknown amino acid residue

<400> 121

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Ser Tyr
20 25 30

Ala Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Ala Ile Ser Trp Ser Gly Thr Ile Thr Asn Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Gly Lys Asn Thr Val His
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr His Cys
85 90 95

Ala Val Val Gln Pro Tyr Ser Gly Gly Asp Tyr Tyr Thr Gly Val Glu
100 105 110

Glu Tyr Asp Tyr Trp Gly Xaa Gly Thr Gln Val Thr Val Ser Ser
115 120 125

<210> 122

<211> 115

<212> PRT

<213> -

<400> 122

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Val Ser Gly Phe Thr Phe Ser Ala Thr
20 25 30

Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Ala Glu Glu Trp Val
35 40 45

Ser Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asp Asp Leu Gln Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Gly Arg Arg Gly Tyr Gly Arg Asp Arg Ser Arg Gly Ile Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 123

<211> 121

<212> PRT

<213> -

<400> 123

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Gly Ala Phe Ser Asn Tyr
20 25 30

Asp Val Gly Trp Phe Arg Gln Ala Pro Gly Glu Gly Arg Glu Ile Val
35 40 45

Ala Arg Ile Ser Gly Ser Gly Asp Ser Thr Tyr Ser Ser Asn Arg Ala
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Arg Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Arg Ala Ala Arg Tyr Asn Gly Thr Trp Ser Ser Asn Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 124

<211> 117

<212> PRT

<213> -

<400> 124

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Glu Cys Val Ser Ser Gly Cys Thr Phe Ser
20 25 30

Ala Tyr Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Ala Glu Glu
35 40 45

Phe Val Ser Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asn
50 55 60

Ser Val Asn Gly Arg Phe Lys Ile Ser Arg Asp Asn Ala Lys Lys Thr
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Gly Pro Glu Asp Thr Ala Met Tyr
85 90 95

Tyr Cys Gln Arg Arg Gly Tyr Ala Leu Asp Arg Gly Gln Gly Thr Gln
100 105 110

Val Thr Val Ser Ser
115

<210> 125

<211> 121

<212> PRT

<213> -

<400> 125

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Gln Thr Ser Ser Thr Ala
20 25 30

Asp Met Gly Trp Phe Arg Gln Pro Pro Gly Lys Gly Arg Glu Phe Val
35 40 45

Ala Arg Ile Ser Gly Ile Asp Gly Thr Thr Tyr Tyr Asp Glu Pro Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Lys Ala Gln Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asp Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Arg Ser Pro Arg Tyr Ala Asp Gln Trp Ser Ala Tyr Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 126

<211> 115

<212> PRT

<213> -

<400> 126

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Val Ser Gly Phe Thr Phe Ser Thr Thr
20 25 30

Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Phe Glu Glu Trp Val
35 40 45

Ser Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Gly Arg Arg Gly Tyr Gly Arg Asp Arg Ser Lys Gly Ile Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 127

<211> 118

<212> PRT

<213> -

<400> 127

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Ala Ser Ala Ser Gly Val Lys Val Asn
20 25 30

Asp Met Gly Trp Tyr Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Val
35 40 45

Ala Thr Ile Thr Asp Asp Gly Arg Thr Asn Tyr Glu Asp Phe Ala Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Leu Pro Glu Asp Thr Ala Val Tyr Tyr Cys Asn
85 90 95

Ala Arg Thr Tyr Trp Ala His Leu Pro Thr Tyr Trp Gly Gln Gly Thr
100 105 110

Gln Val Thr Val Ser Ser
115

<210> 128

<211> 121

<212> PRT

<213> -

<400> 128

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Phe Gly Ser Val
20 25 30

Ala Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Ala Ile Gly Tyr Asp Gly Asn Ser Ile Arg Tyr Gly Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ile Lys Asn Thr Met Tyr
65 70 75 80

Leu Glu Met Glu Asn Leu Asn Ala Asp Asp Thr Ala Arg Tyr Leu Cys
85 90 95

Ala Ala Glu Pro Leu Ala Arg Tyr Glu Gly Leu Trp Thr Tyr Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 129

<211> 121

<212> PRT

<213> -

<400> 129

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Ala
1 5 10 15

Ser Leu Arg Leu Ser Cys Thr Thr Ser Thr Arg Thr Asn Asp Arg Phe
20 25 30

Asn Met Ala Trp Phe His Gln Ala Pro Gly Lys Asp Arg Glu Phe Val
35 40 45

Ser Arg Ile Asp Val Ala Gly Tyr Asn Thr Ala Tyr Gly Asp Phe Val
50 55 60

Lys Gly Arg Phe Thr Val Ser Arg Asp Ser Ala Glu Asn Thr Val Val
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Tyr Cys
85 90 95

Ala Ala Gly Gly Trp Gly Ile Ser Gln Ser Asp Tyr Asp Leu Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 130

<211> 30

<212> PRT

<213> -

<400> 130

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
20 25 30

<210> 131

<211> 30

<212> PRT

<213> -

<400> 131

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Met Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Gly
20 25 30

<210> 132

<211> 30

<212> PRT

<213> -

<400> 132

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Asp Phe Ser
20 25 30

<210> 133

<211> 30

<212> PRT

<213> -

<400> 133

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser
20 25 30

<210> 134

<211> 30

<212> PRT

<213> -

<400> 134

Gln Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser
20 25 30

<210> 135

<211> 30

<212> PRT

<213> -

<400> 135

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser
20 25 30

<210> 136

<211> 30

<212> PRT

<213> -

<400> 136

Ala Val Gln Leu Val Asp Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser
20 25 30

<210> 137

<211> 30

<212> PRT

<213> -

<400> 137

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ala Ala Ser Gly Arg Ser Phe Thr
20 25 30

<210> 138

<211> 30

<212> PRT

<213> -

<400> 138

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser
20 25 30

<210> 139

<211> 30

<212> PRT

<213> -

<400> 139

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gln Ile Ile Phe Gly
20 25 30

<210> 140

<211> 30

<212> PRT

<213> -

<400> 140

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Asn Ala Gly Ser Thr Ser Asn
20 25 30

<210> 141

<211> 30

<212> PRT

<213> -

<400> 141

Glu Val Gln Leu Val Glu Ser Gly Gly Asp Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Gln Leu Phe Ser
20 25 30

<210> 142

<211> 30

<212> PRT

<213> -

<400> 142

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Val Ser Gly Phe Thr Phe Ser
20 25 30

<210> 143

<211> 30

<212> PRT

<213> -

<400> 143

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Thr Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Phe Ser
20 25 30

<210> 144

<211> 30

<212> PRT

<213> -

<220>

<221> misc_feature

<222> (1)..(4)

<223> Unknown amino acid residues, probably EVQL, QVQL or a similar seq
uence

<400> 144

Xaa Xaa Xaa Xaa Val Glu Ser Gly Gly Gly Leu Met Gln Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Met Phe Ser
20 25 30

<210> 145

<211> 30

<212> PRT

<213> -

<400> 145

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Gly
20 25 30

<210> 146

<211> 30

<212> PRT

<213> -

<400> 146

Glu Val Gln Leu Val Glu Ser Gly Gly Glu Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Arg Asn Phe Val
20 25 30

<210> 147

<211> 30

<212> PRT

<213> -

<400> 147

Glu Val Gln Leu Val Glu Ser Gly Gly Arg Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Asn Ala Gly Ser Thr Ser Asn
20 25 30

<210> 148

<211> 30

<212> PRT

<213> -

<400> 148

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Ala Thr Ile Ser Ser
20 25 30

<210> 149

<211> 30

<212> PRT

<213> -

<400> 149

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Gln Thr Ser Ser
20 25 30

<210> 150

<211> 30

<212> PRT

<213> -

<400> 150

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ser Thr Phe Ser
20 25 30

<210> 151

<211> 30

<212> PRT

<213> -

<400> 151

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Glu Ala Ser Glu Arg Ser Ser Asn
20 25 30

<210> 152

<211> 30

<212> PRT

<213> -

<400> 152

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Cys Val Ser Ser Gly Cys Thr
20 25 30

<210> 153

<211> 30

<212> PRT

<213> -

<400> 153

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Thr Leu Ser Cys Ala Ser Ser Gly Arg Gly Phe Tyr
20 25 30

<210> 154

<211> 30

<212> PRT

<213> -

<400> 154

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Phe Ser Gly Phe Ala Phe Ser
20 25 30

<210> 155

<211> 30

<212> PRT

<213> -

<400> 155

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser
20 25 30

<210> 156

<211> 30

<212> PRT

<213> -

<400> 156

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Val Ser Gly Phe Thr Phe Ser
20 25 30

<210> 157

<211> 30

<212> PRT

<213> -

<400> 157

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Gly Ala Phe Ser
20 25 30

<210> 158

<211> 30

<212> PRT

<213> -

<400> 158

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Glu Cys Val Ser Ser Gly Cys Thr
20 25 30

<210> 159

<211> 30

<212> PRT

<213> -

<400> 159

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Gln Thr Ser Ser
20 25 30

<210> 160

<211> 30

<212> PRT

<213> -

<400> 160

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Val Ser Gly Phe Thr Phe Ser
20 25 30

<210> 161

<211> 30

<212> PRT

<213> -

<400> 161

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Ala Ser Ala Ser Gly Val Lys
20 25 30

<210> 162

<211> 30

<212> PRT

<213> -

<400> 162

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Phe Gly
20 25 30

<210> 163

<211> 29

<212> PRT

<213> -

<400> 163

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Ala
1 5 10 15

Ser Leu Arg Leu Ser Cys Thr Thr Ser Thr Arg Thr Asn
20 25

<210> 164

<211> 5

<212> PRT

<213> -

<400> 164

Asp Tyr Trp Met Tyr
1 5

<210> 165

<211> 5

<212> PRT

<213> -

<400> 165

Val Ser Trp Met Tyr
1 5

<210> 166

<211> 5

<212> PRT

<213> -

<400> 166

Val Ser Trp Met Tyr
1 5

<210> 167

<211> 10

<212> PRT

<213> -

<400> 167

Glu Pro Ser Gly Tyr Thr Tyr Thr Ile Gly
1 5 10

<210> 168

<211> 10

<212> PRT

<213> -

<400> 168

Asp Tyr Ser Gly Tyr Thr Tyr Thr Val Gly
1 5 10

<210> 169

<211> 10

<212> PRT

<213> -

<400> 169

Asp Tyr Ser Gly Tyr Thr Tyr Thr Val Gly
1 5 10

<210> 170

<211> 8

<212> PRT

<213> -

<400> 170

Ala His Ser Val Tyr Thr Met Gly
1 5

<210> 171

<211> 5

<212> PRT

<213> -

<400> 171

Gly Tyr Tyr Met Gly
1 5

<210> 172

<211> 5

<212> PRT

<213> -

<400> 172

Asn Tyr Tyr Met Gly
1 5

<210> 173

<211> 5

<212> PRT

<213> -

<400> 173

Ser His Val Ala Ala
1 5

<210> 174

<211> 5

<212> PRT

<213> -

<400> 174

Ala Tyr Ala Thr Gly
1 5

<210> 175

<211> 5

<212> PRT

<213> -

<400> 175

Thr Asn Asp Val Gly
1 5

<210> 176

<211> 5

<212> PRT

<213> -

<400> 176

Thr Thr Ser Met Thr
1 5

<210> 177

<211> 5

<212> PRT

<213> -

<400> 177

Ser Val Ala Met Gly
1 5

<210> 178

<211> 5

<212> PRT

<213> -

<400> 178

Asp Ser Ala Met Gly
1 5

<210> 179

<211> 5

<212> PRT

<213> -

<400> 179

Thr Tyr Ala Met Gly
1 5

<210> 180

<211> 5

<212> PRT

<213> -

<400> 180

Thr Tyr Ala Met Ser
1 5

<210> 181

<211> 5

<212> PRT

<213> -

<400> 181

Ala Tyr Ala Thr Gly
1 5

<210> 182

<211> 5

<212> PRT

<213> -

<400> 182

Ile Val Met Leu Gly
1 5

<210> 183

<211> 5

<212> PRT

<213> -

<400> 183

Ser Tyr Asp Met Gly
1 5

<210> 184

<211> 5

<212> PRT

<213> -

<400> 184

Thr Tyr Asp Met Ser
1 5

<210> 185

<211> 5

<212> PRT

<213> -

<400> 185

Arg Tyr Asn Met Ala
1 5

<210> 186

<211> 7

<212> PRT

<213> -

<400> 186

Phe Ser Ala Tyr Ser Met Thr
1 5

<210> 187

<211> 5

<212> PRT

<213> -

<400> 187

Lys Asn Ala Met Gly
1 5

<210> 188

<211> 5

<212> PRT

<213> -

<400> 188

Ala Ser Ser Met Ala
1 5

<210> 189

<211> 5

<212> PRT

<213> -

<400> 189

Ser Tyr Ala Met Gly
1 5

<210> 190

<211> 5

<212> PRT

<213> -

<400> 190

Ala Thr Ser Met Thr
1 5

<210> 191

<211> 5

<212> PRT

<213> -

<400> 191

Asn Tyr Asp Val Gly
1 5

<210> 192

<211> 7

<212> PRT

<213> -

<400> 192

Phe Ser Ala Tyr Ser Met Thr
1 5

<210> 193

<211> 5

<212> PRT

<213> -

<400> 193

Thr Ala Asp Met Gly
1 5

<210> 194

<211> 5

<212> PRT

<213> -

<400> 194

Thr Thr Ser Met Thr
1 5

<210> 195

<211> 5

<212> PRT

<213> -

<400> 195

Val Asn Asp Met Gly
1 5

<210> 196

<211> 5

<212> PRT

<213> -

<400> 196

Ser Val Ala Met Gly
1 5

<210> 197

<211> 6

<212> PRT

<213> -

<400> 197

Asp Arg Phe Asn Met Ala
1 5

<210> 198

<211> 14

<212> PRT

<213> -

<400> 198

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
1 5 10

<210> 199

<211> 14

<212> PRT

<213> -

<400> 199

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
1 5 10

<210> 200

<211> 14

<212> PRT

<213> -

<400> 200

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
1 5 10

<210> 201

<211> 14

<212> PRT

<213> -

<400> 201

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

399

<210> 202

<211> 14

<212> PRT

<213> -

<400> 202

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 203

<211> 14

<212> PRT

<213> -

<400> 203

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 204

<211> 14

<212> PRT

<213> -

<400> 204

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 205

<211> 14

<212> PRT

<213> -

<400> 205

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Gln Leu Leu Ala
1 5 10

<210> 206

<211> 14

<212> PRT

<213> -

<400> 206

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu Gly
1 5 10

<210> 207

<211> 14

<212> PRT

<213> -

<400> 207

Trp Phe Arg Gln Ala Pro Gly Arg Glu Arg Glu Phe Val Ala
1 5 10

<210> 208

<211> 14

<212> PRT

<213> -

<400> 208

Trp Phe Arg Arg Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 209

<211> 14

<212> PRT

<213> -

<400> 209

Trp Tyr Arg Arg Ala Pro Gly Lys Gln Arg Glu Leu Val Ala
1 5 10

<210> 210

<211> 14

<212> PRT

<213> -

<400> 210

Trp Val Arg Gln Ala Pro Gly Lys Phe Glu Glu Trp Val Ser
1 5 10

<210> 211

<211> 14

<212> PRT

<213> -

<400> 211

Trp Phe Arg Gln Ala Pro Gly Lys Gln Arg Glu Phe Leu Ala
1 5 10

<210> 212

<211> 14

<212> PRT

<213> -

<400> 212

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 213

<211> 14

<212> PRT

<213> -

<400> 213

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 214

<211> 14

<212> PRT

<213> -

<400> 214

Trp Phe Arg Arg Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 215

<211> 14

<212> PRT

<213> -

<400> 215

Trp Phe Arg Arg Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 216

<211> 14

<212> PRT

<213> -

<400> 216

Trp Tyr Arg Gln Ala Pro Gly Lys Gln Arg Glu Trp Val Ala
1 5 10

403

<210> 217

<211> 14

<212> PRT

<213> -

<400> 217

Trp Phe Arg Gln Ala Pro Gly Glu Gly Arg Glu Phe Val Ala
1 5 10

<210> 218

<211> 14

<212> PRT

<213> -

<400> 218

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
1 5 10

<210> 219

<211> 14

<212> PRT

<213> -

<400> 219

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Leu Ala
1 5 10

<210> 220

<211> 14

<212> PRT

<213> -

<400> 220

Trp Val Arg Gln Ala Pro Gly Lys Ala Glu Glu Trp Val Ser
1 5 10

<210> 221

<211> 14

<212> PRT

<213> -

<400> 221

Trp Phe Arg Gln Pro Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 222

<211> 14

<212> PRT

<213> -

<400> 222

Trp Val Arg Gln Ala Pro Gly Lys Tyr Glu Glu Trp Val Ser
1 5 10

<210> 223

<211> 14

<212> PRT

<213> -

<400> 223

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 224

<211> 14

<212> PRT

<213> -

405

<400> 224

Trp Val Arg Gln Ala Pro Gly Lys Ala Glu Glu Trp Val Ser
1 5 10

<210> 225

<211> 14

<212> PRT

<213> -

<400> 225

Trp Phe Arg Gln Ala Pro Gly Glu Gly Arg Glu Ile Val Ala
1 5 10

<210> 226

<211> 14

<212> PRT

<213> -

<400> 226

Trp Val Arg Gln Ala Pro Gly Lys Ala Glu Glu Phe Val Ser
1 5 10

<210> 227

<211> 14

<212> PRT

<213> -

<400> 227

Trp Phe Arg Gln Pro Pro Gly Lys Gly Arg Glu Phe Val Ala
1 5 10

<210> 228

<211> 14

<212> PRT

<213> -

<400> 228

Trp Val Arg Gln Ala Pro Gly Lys Phe Glu Glu Trp Val Ser
1 5 10

<210> 229

<211> 14

<212> PRT

<213> -

<400> 229

Trp Tyr Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Val Ala
1 5 10

<210> 230

<211> 14

<212> PRT

<213> -

<400> 230

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 231

<211> 14

<212> PRT

<213> -

<400> 231

Trp Phe His Gln Ala Pro Gly Lys Asp Arg Glu Phe Val Ser
1 5 10

<210> 232

<211> 17

<212> PRT

<213> -

<400> 232

Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val Lys
1 5 10 15

Gly

<210> 233

<211> 17

<212> PRT

<213> -

<400> 233

Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val Lys
1 5 10 15

Gly

<210> 234

<211> 17

<212> PRT

<213> -

<400> 234

Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Val Asp Ser Val Lys
1 5 10 15

Gly

<210> 235

<211> 17

<212> PRT

<213> -

<400> 235

Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 236

<211> 17

<212> PRT

<213> -

<400> 236

Arg Ile Tyr Trp Ser Ser Gly Asn Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 237

<211> 17

<212> PRT

<213> -

<400> 237

Arg Ile Tyr Trp Ser Ser Gly Asn Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

409

<210> 238

<211> 17

<212> PRT

<213> -

<400> 238

Arg Ile Tyr Trp Ser Ser Ala Asn Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 239

<211> 17

<212> PRT

<213> -

<400> 239

Ser Ile Ser Trp Arg Gly Asp Asn Thr Tyr Tyr Lys Glu Ser Val Lys
1 5 10 15

Gly

<210> 240

<211> 17

<212> PRT

<213> -

<400> 240

Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val Lys
1 5 10 15

Gly

<210> 241

<211> 23

<212> PRT

<213> -

<400> 241

Glu Ile Arg Pro Ser Gly Asp Phe Gly Pro Glu Gly Glu Phe Glu His
1 5 10 15

Val Thr Ala Ser Leu Lys Gly
20

<210> 242

<211> 17

<212> PRT

<213> -

<400> 242

Gly Ile Gln Trp Ser Gly Gly Asp Ala Phe Tyr Arg Asn Ser Val Lys
1 5 10 15

Gly

<210> 243

<211> 16

<212> PRT

<213> -

<400> 243

Thr Ile Thr Asp Asp Gly Thr Thr Asp Tyr Gly Asp Asp Val Lys Gly
1 5 10 15

<210> 244

<211> 17

<212> PRT

<213> -

<400> 244

Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 245

<211> 17

<212> PRT

<213> -

<400> 245

Gly Val Gly Tyr Asp Gly Ser Ser Ile Arg Tyr Ala Glu Ser Val Lys
1 5 10 15

Gly

<210> 246

<211> 17

<212> PRT

<213> -

<400> 246

Thr Ile Ser Trp Asn Gly Gly Ser Ser Ser Tyr Ala Asp Phe Val Lys
1 5 10 15

Gly

<210> 247

<211> 17

<212> PRT

<213> -

<400> 247

Ala Ile Ser Trp Gly Gly Gly Ser Ile Val Tyr Ala Glu Ser Ala Lys
1 5 10 15

Gly

<210> 248

<211> 17

<212> PRT

<213> -

<400> 248

Ser Ile Ser Trp Ser Gly Asp Thr Thr Tyr Tyr Ser Asn Ser Val Lys
1 5 10 15

Gly

<210> 249

<211> 17

<212> PRT

<213> -

<400> 249

Gly Ile Gln Trp Ser Gly Gly Asp Ala Phe Tyr Arg Asn Ser Val Lys
1 5 10 15

Gly

<210> 250

<211> 16

<212> PRT

<213> -

<400> 250

Ser Ile Thr Ile Gly Ser Arg Thr Asn Tyr Ala Asp Ser Val Lys Gly
1 5 10 15

<210> 251

<211> 17

<212> PRT

<213> -

<400> 251

Arg Ile Ser Gly Ser Asp Gly Ser Thr Tyr Tyr Ser Asp Arg Ala Lys
1 5 10 15

Asp

<210> 252

<211> 17

<212> PRT

<213> -

<400> 252

Gly Ile Asp Ser Gly Gly Gly Ser Pro Met Tyr Val Asp Ser Val Lys
1 5 10 15

Gly

<210> 253

<211> 17

<212> PRT

<213> -

414

<400> 253

Arg Val Asp Val Ser Gly Gly Asn Thr Leu Tyr Gly Asp Ser Val Lys
1 5 10 15

Asp

<210> 254

<211> 17

<212> PRT

<213> -

<400> 254

Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val Asn
1 5 10 15

Gly

<210> 255

<211> 17

<212> PRT

<213> -

<400> 255

Ser Ile Lys Trp Asn Gly Asn Asn Thr Tyr Tyr Ala Asp Ser Val Arg
1 5 10 15

Gly

<210> 256

<211> 17

<212> PRT

<213> -

<400> 256

Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val Gln
1 5 10 15

Gly

<210> 257

<211> 17

<212> PRT

<213> -

<400> 257

Ala Ile Ser Trp Ser Gly Thr Ile Thr Asn Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 258

<211> 17

<212> PRT

<213> -

<400> 258

Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 259

<211> 17

<212> PRT

<213> -

416

<400> 259

Arg Ile Ser Gly Ser Gly Asp Ser Thr Tyr Ser Ser Asn Arg Ala Lys
1 5 10 15

Gly

<210> 260

<211> 17

<212> PRT

<213> -

<400> 260

Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asn Ser Val Asn
1 5 10 15

Gly

<210> 261

<211> 17

<212> PRT

<213> -

<400> 261

Arg Ile Ser Gly Ile Asp Gly Thr Thr Tyr Tyr Asp Glu Pro Val Lys
1 5 10 15

Gly

<210> 262

<211> 17

<212> PRT

<213> -

<400> 262

Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 263

<211> 16

<212> PRT

<213> -

<400> 263

Thr Ile Thr Asp Asp Gly Arg Thr Asn Tyr Glu Asp Phe Ala Lys Gly
1 5 10 15

<210> 264

<211> 17

<212> PRT

<213> -

<400> 264

Ala Ile Gly Tyr Asp Gly Asn Ser Ile Arg Tyr Gly Asp Ser Val Lys
1 5 10 15

Gly

<210> 265

<211> 17

<212> PRT

<213> -

<400> 265

Arg Ile Asp Val Ala Gly Tyr Asn Thr Ala Tyr Gly Asp Phe Val Lys
1 5 10 15

Gly

<210> 266

<211> 32

<212> PRT

<213> -

<400> 266

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg
20 25 30

<210> 267

<211> 32

<212> PRT

<213> -

<400> 267

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg
20 25 30

<210> 268

<211> 32

<212> PRT

<213> -

<400> 268

419

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asp Ser Leu Ile Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg
20 25 30

<210> 269

<211> 32

<212> PRT

<213> -

<400> 269

Arg Phe Thr Ile Ser Arg Asp Ile Ala Lys Asn Thr Val Asp Leu Leu
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 270

<211> 32

<212> PRT

<213> -

<400> 270

Arg Phe Thr Ile Ser Arg Asp Ile Ala Lys Asn Thr Val Asp Leu Leu
1 5 10 15

Met Asn Asn Leu Glu Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 271

<211> 32

<212> PRT

<213> -

<400> 271

Arg Phe Thr Ile Ser Arg Asp Ile Ala Lys Asn Thr Val Asp Leu Leu
1 5 10 15

Met Asn Asn Leu Glu Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 272

<211> 32

<212> PRT

<213> -

<400> 272

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Asp Leu Leu
1 5 10 15

Met Asn Cys Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 273

<211> 32

<212> PRT

<213> -

<400> 273

Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 274

<211> 32

<212> PRT

<213> -

<400> 274

Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr Leu Gln
1 5 10 15

Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 275

<211> 32

<212> PRT

<213> -

<400> 275

Arg Phe Thr Ile Ala Lys Asn Ser Val Asp Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 276

<211> 31

<212> PRT

<213> -

<400> 276

Arg Phe Arg Ile Thr Arg Asp Pro Asp Asn Thr Val Tyr Leu Gln Met
1 5 10 15

Asn Asp Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr Cys Ala Gln
20 25 30

<210> 277

<211> 30

<212> PRT

<213> -

<400> 277

Arg Phe Val Ile Ser Arg Glu Gly Glu Met Val Tyr Leu Glu Met Asn
1 5 10 15

Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Asn Ile
20 25 30

<210> 278

<211> 32

<212> PRT

<213> -

<400> 278

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Gly Arg
20 25 30

<210> 279

<211> 32

<212> PRT

<213> -

<400> 279

Arg Phe Thr Ile Ala Arg Gly Asn Arg Glu Ser Thr Val Phe Leu Gln
1 5 10 15

Met Glu Asn Leu Lys Pro Glu Asp Thr Ala Val Tyr Phe Cys Thr Ala
20 25 30

<210> 280

<211> 32

<212> PRT

<213> -

<400> 280

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Gly Leu Thr Pro Gln Asp Thr Ala Ile Tyr Tyr Cys Ala Gly
20 25 30

<210> 281

<211> 32

<212> PRT

<213> -

<220>

<221> misc_feature

<222> (11)..(11)

<223> unknown amino acid residue

<400> 281

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Xaa Thr Met Tyr Leu Gln
1 5 10 15

Met Asp Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 282

<211> 32

<212> PRT

<213> -

<400> 282

Arg Phe Thr Val Ser Arg Asp Asn Gly Lys Asn Thr Ala Tyr Leu Arg
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Asp Tyr Tyr Cys Ala Val
20 25 30

<210> 283

<211> 31

<212> PRT

<213> -

<400> 283

Arg Phe Arg Ile Thr Arg Asp Pro Asp Asn Thr Val Tyr Leu Gln Met
1 5 10 15

Asn Asp Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr Cys Ala Gln
20 25 30

<210> 284

<211> 32

<212> PRT

<213> -

<400> 284

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Phe Cys Asn Ala
20 25 30

<210> 285

<211> 32

<212> PRT

<213> -

<400> 285

Arg Phe Thr Ile Ser Arg Asp Asn Thr Lys Asn Met Val Tyr Leu Gln
1 5 10 15

Met Asp Arg Leu Lys Pro Asp Asp Thr Ala Val Tyr Tyr Cys Arg Val
20 25 30

<210> 286

<211> 32

<212> PRT

<213> -

<400> 286

Arg Phe Thr Val Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys
20 25 30

<210> 287

<211> 32

<212> PRT

<213> -

<400> 287

Arg Phe Thr Val Ser Arg Ile Asn Gly Lys Asn Ala Met Tyr Leu Gln
1 5 10 15

Met Asn Asn Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr Cys Ala Ala
20 25 30

<210> 288

<211> 32

<212> PRT

<213> -

<400> 288

Arg Phe Lys Ile Ser Arg Asp Asn Ala Lys Lys Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Gly Pro Glu Asp Thr Ala Met Tyr Tyr Cys Gln Arg
20 25 30

<210> 289

<211> 35

<212> PRT

<213> -

<400> 289

Arg Phe Thr Ile Ser Arg Gly Asn Ala Lys Asn Thr Glu Asn Thr Val
1 5 10 15

Ser Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Asp Tyr Tyr
20 25 30

Cys Ala Ala
35

<210> 290

<211> 32

<212> PRT

<213> -

<400> 290

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys Gly Arg
20 25 30

<210> 291

<211> 32

<212> PRT

<213> -

<400> 291

Arg Phe Thr Ile Ser Arg Asp Asn Gly Lys Asn Thr Val His Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr His Cys Ala Val
20 25 30

<210> 292

<211> 32

<212> PRT

<213> -

<400> 292

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asp Asp Leu Gln Ser Glu Asp Thr Ala Met Tyr Tyr Cys Gly Arg
20 25 30

<210> 293

<211> 32

<212> PRT

<213> -

<400> 293

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Arg Glu Asp Thr Ala Val Tyr Tyr Cys Arg Ala
20 25 30

<210> 294

<211> 32

<212> PRT

<213> -

<400> 294

Arg Phe Lys Ile Ser Arg Asp Asn Ala Lys Lys Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Gly Pro Glu Asp Thr Ala Met Tyr Tyr Cys Gln Arg
20 25 30

<210> 295

<211> 32

<212> PRT

<213> -

<400> 295

Arg Phe Thr Ile Ser Arg Asp Lys Ala Gln Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asp Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Arg Ser
20 25 30

<210> 296

<211> 32

<212> PRT

<213> -

<400> 296

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Gly Arg
20 25 30

<210> 297

<211> 32

<212> PRT

<213> -

<400> 297

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Leu Pro Glu Asp Thr Ala Val Tyr Tyr Cys Asn Ala
20 25 30

<210> 298

<211> 32

<212> PRT

<213> -

<400> 298

Arg Phe Thr Ile Ser Arg Asp Asn Ile Lys Asn Thr Met Tyr Leu Glu
1 5 10 15

Met Glu Asn Leu Asn Ala Asp Asp Thr Ala Arg Tyr Leu Cys Ala Ala
20 25 30

<210> 299

<211> 32

<212> PRT

<213> -

<400> 299

Arg Phe Thr Val Ser Arg Asp Ser Ala Glu Asn Thr Val Val Leu Gln
1 5 10 15

Met Asn Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 300

<211> 6

<212> PRT

<213> -

<400> 300

Ser Pro Ser Gly Phe Asn
1 5

<210> 301

<211> 6

<212> PRT

<213> -

<400> 301

Ser Pro Ser Gly Ser Phe
1 5

<210> 302

<211> 6

<212> PRT

<213> -

<400> 302

Ser Pro Ser Gly Ser Phe
1 5

<210> 303

<211> 15

<212> PRT

<213> -

<400> 303

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Gly Ser Tyr Asn Tyr
1 5 10 15

<210> 304

<211> 15

<212> PRT

<213> -

<400> 304

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Glu Ser Tyr Asn Tyr
1 5 10 15

<210> 305

<211> 15

<212> PRT

<213> -

<400> 305

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Glu Ser Tyr Asn Tyr
1 5 10 15

<210> 306

<211> 15

<212> PRT

<213> -

<400> 306

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Glu Ala Tyr Asn Tyr
1 5 10 15

<210> 307

<211> 14

<212> PRT

<213> -

<400> 307

Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Asn
1 5 10

<210> 308

<211> 14

<212> PRT

<213> -

<400> 308

Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
1 5 10

<210> 309

<211> 17

<212> PRT

<213> -

<400> 309

Ala Pro Tyr Arg Gly Gly Arg Asp Tyr Arg Trp Glu Tyr Glu Tyr Glu
1 5 10 15

Tyr

<210> 310

<211> 16

<212> PRT

<213> -

<400> 310

Lys Leu Ser Pro Tyr Tyr Asn Asp Phe Asp Ser Ser Asn Tyr Glu Tyr
1 5 10 15

<210> 311

<211> 13

<212> PRT

<213> -

<400> 311

Asn Arg Leu Arg Ser Thr Trp Gly Ile Arg Tyr Asp Val
1 5 10

<210> 312

<211> 6

<212> PRT

<213> -

<400> 312

Arg Gly Tyr Gly Arg Asp
1 5

<210> 313

<211> 12

<212> PRT

<213> -

<400> 313

Glu Pro Ile Gly Ala Tyr Glu Gly Leu Trp Thr Tyr
1 5 10

<210> 314

<211> 15

<212> PRT

<213> -

<400> 314

Ser Tyr Ser Asn Gly Asn Pro His Arg Phe Ser Gln Tyr Gln Tyr
1 5 10 15

<210> 315

<211> 11

<212> PRT

<213> -

<400> 315

Ala Asn Asn Ile Ala Thr Leu Arg Gln Gly Ser
1 5 10

<210> 316

<211> 18

<212> PRT

<213> -

<400> 316

Val Gln Val Ile Asp Pro Ser Trp Ser Gly Val Asn Leu Asp Asp Tyr
1 5 10 15

Asp Tyr

<210> 317

<211> 16

<212> PRT

<213> -

<400> 317

Lys Leu Ser Pro Tyr Tyr Asn Asp Phe Asp Ser Ser Asn Tyr Glu Tyr
1 5 10 15

<210> 318

<211> 7

<212> PRT

<213> -

<400> 318

Val Pro Pro Arg Asp Asp Tyr
1 5

<210> 319

<211> 12

<212> PRT

<213> -

<400> 319

Pro Arg Tyr Glu Asn Gln Trp Ser Ser Tyr Asp Tyr
1 5 10

<210> 320

<211> 18

<212> PRT

<213> -

<400> 320

Phe Ser Thr Gly Ala Asp Gly Gly Ser Trp Tyr Trp Ser Tyr Gly Met
1 5 10 15

Asp Ser

<210> 321

<211> 12

<212> PRT

<213> -

<400> 321

Gly Gly Trp Gly Thr Thr Gln Tyr Asp Tyr Asp Tyr
1 5 10

<210> 322

<211> 6

<212> PRT

<213> -

<400> 322

Arg Gly Tyr Ala Leu Asp
1 5

<210> 323

<211> 17

<212> PRT

<213> -

<400> 323

Asp Ser Ser His Tyr Ser Tyr Val Tyr Ser Lys Ala Tyr Glu Tyr Asp
1 5 10 15

Tyr

<210> 324

<211> 6

<212> PRT

<213> -

<400> 324

Arg Gly Tyr Gly Arg Asp
1 5

<210> 325

<211> 18

<212> PRT

<213> -

<400> 325

Val Gln Pro Tyr Ser Gly Gly Asp Tyr Tyr Thr Gly Val Glu Glu Tyr
1 5 10 15

Asp Tyr

<210> 326

<211> 6

<212> PRT

<213> -

<400> 326

Arg Gly Tyr Gly Arg Asp
1 5

<210> 327

<211> 12

<212> PRT

<213> -

<400> 327

Ala Arg Tyr Asn Gly Thr Trp Ser Ser Asn Asp Tyr
1 5 10

<210> 328

<211> 6

<212> PRT

<213> -

<400> 328

Arg Gly Tyr Ala Leu Asp
1 5

<210> 329

<211> 12

<212> PRT

<213> -

<400> 329

Pro Arg Tyr Ala Asp Gln Trp Ser Ala Tyr Asp Tyr
1 5 10

<210> 330

<211> 6

<212> PRT

<213> -

<400> 330

Arg Gly Tyr Gly Arg Asp
1 5

<210> 331

<211> 10

<212> PRT

<213> -

<400> 331

Arg Thr Tyr Trp Ala His Leu Pro Thr Tyr
1 5 10

<210> 332

<211> 12

<212> PRT

<213> -

<400> 332

Glu Pro Leu Ala Arg Tyr Glu Gly Leu Trp Thr Tyr
1 5 10

<210> 333

<211> 12

<212> PRT

<213> -

<400> 333

Gly Gly Trp Gly Ile Ser Gln Ser Asp Tyr Asp Leu
1 5 10

<210> 334

<211> 11

<212> PRT

<213> -

<400> 334

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 335

<211> 11

<212> PRT

<213> -

<400> 335

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 336

<211> 11

<212> PRT

<213> -

<400> 336

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 337

<211> 11

<212> PRT

<213> -

<400> 337

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 338

<211> 11

<212> PRT

<213> -

<400> 338

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 339

<211> 11

<212> PRT

<213> -

<400> 339

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 340

<211> 11

<212> PRT

<213> -

<400> 340

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 341

<211> 11

<212> PRT

<213> -

<400> 341

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 342

<211> 11

<212> PRT

<213> -

<400> 342

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 343

<211> 11

<212> PRT

<213> -

<400> 343

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 344

<211> 11

<212> PRT

<213> -

<400> 344

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 345

<211> 11

<212> PRT

<213> -

<400> 345

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 346

<211> 11

<212> PRT

<213> -

<400> 346

Arg Ser Lys Gly Ile Gln Val Thr Val Ala Ser
1 5 10

<210> 347

<211> 11

<212> PRT

<213> -

<400> 347

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 348

<211> 11

<212> PRT

<213> -

<400> 348

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 349

<211> 11

<212> PRT

<213> -

<400> 349

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 350

<211> 11

<212> PRT

<213> -

<400> 350

Leu Gly Ser Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 351

<211> 11

<212> PRT

<213> -

<400> 351

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 352

<211> 11

<212> PRT

<213> -

<400> 352

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 353

<211> 11

<212> PRT

<213> -

<400> 353

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 354

<211> 11

<212> PRT

<213> -

<400> 354

Trp Gly Lys Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 355

<211> 11

<212> PRT

<213> -

<400> 355

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 356

<211> 11

<212> PRT

<213> -

<400> 356

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 357

<211> 11

<212> PRT

<213> -

<400> 357

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 358

<211> 11

<212> PRT

<213> -

<400> 358

Arg Ser Gln Gly Ile Gln Val Thr Val Ser Ser
1 5 10

<210> 359

<211> 11

<212> PRT

<213> -

<220>

<221> misc_feature

<222> (3)..(3)

<223> Unknown amino acid residue, probably Q

<400> 359

Trp Gly Xaa Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 360

<211> 11

<212> PRT

<213> -

<400> 360

Arg Ser Arg Gly Ile Gln Val Thr Val Ser Ser
1 5 10

<210> 361

<211> 11

<212> PRT

<213> -

<400> 361

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 362

<211> 11

<212> PRT

<213> -

<400> 362

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 363

<211> 11

<212> PRT

<213> -

<400> 363

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 364

<211> 11

<212> PRT

<213> -

<400> 364

Arg Ser Lys Gly Ile Gln Val Thr Val Ser Ser
1 5 10

<210> 365

<211> 11

<212> PRT

<213> -

<400> 365

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 366

<211> 11

<212> PRT

<213> -

<400> 366

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 367

<211> 11

<212> PRT

<213> -

<400> 367

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 368

<211> 30

<212> PRT

<213> -

<400> 368

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Gly Gly Gly
1 5 10 15

Ser Leu Arg Leu Ala Cys Ala Ala Ser Glu Arg Ile Phe Asp
20 25 30

<210> 369

<211> 30

<212> PRT

<213> -

<400> 369

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Glu Gly Gly
1 5 10 15

Ser Leu Arg Leu Ala Cys Ala Ala Ser Glu Arg Ile Trp Asp
20 25 30

<210> 370

<211> 30

<212> PRT

<213> -

<400> 370

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg
20 25 30

<210> 371

<211> 30

<212> PRT

<213> -

<400> 371

Ala Val Gln Leu Val Asp Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Phe Gly
20 25 30

<210> 372

<211> 30

<212> PRT

<213> -

<400> 372

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Thr Cys Thr Ala Ser Gly Phe Thr Phe Arg
20 25 30

<210> 373

<211> 30

<212> PRT

<213> -

<400> 373

Gln Val Gln Leu Ala Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Thr Cys Thr Ala Ser Gly Phe Thr Phe Gly
20 25 30

<210> 374

<211> 30

<212> PRT

<213> -

<400> 374

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
20 25 30

<210> 375

<211> 5

<212> PRT

<213> -

<400> 375

Leu Asn Leu Met Gly
1 5

<210> 376

<211> 5

<212> PRT

<213> -

<400> 376

Ile Asn Leu Leu Gly
1 5

<210> 377

<211> 5

<212> PRT

<213> -

<400> 377

Ser Phe Gly Met Ser
1 5

<210> 378

<211> 5

<212> PRT

<213> -

<400> 378

Ser Phe Gly Met Ser
1 5

<210> 379

<211> 5

<212> PRT

<213> -

<400> 379

Ser Phe Gly Met Ser
1 5

452

<210> 380

<211> 5

<212> PRT

<213> -

<400> 380

Ser Phe Gly Met Ser
1 5

<210> 381

<211> 5

<212> PRT

<213> -

<400> 381

Asn Tyr Trp Met Tyr
1 5

<210> 382

<211> 14

<212> PRT

<213> -

<400> 382

Trp Tyr Arg Gln Gly Pro Gly Asn Glu Arg Glu Leu Val Ala
1 5 10

<210> 383

<211> 14

<212> PRT

<213> -

<400> 383

Trp Tyr Arg Gln Gly Pro Gly Asn Glu Arg Glu Leu Val Ala
1 5 10

<210> 384

<211> 14

<212> PRT

<213> -

<400> 384

Trp Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val Ser
1 5 10

<210> 385

<211> 14

<212> PRT

<213> -

<400> 385

Trp Val Arg Gln Tyr Pro Gly Lys Glu Pro Glu Trp Val Ser
1 5 10

<210> 386

<211> 14

<212> PRT

<213> -

<400> 386

Trp Val Arg Gln Ala Pro Gly Lys Asp Gln Glu Trp Val Ser
1 5 10

<210> 387

<211> 14

<212> PRT

<213> -

<400> 387

Trp Val Arg Gln Ala Pro Gly Glu Gly Leu Glu Trp Val Ser
1 5 10

<210> 388

<211> 14

<212> PRT

<213> -

<400> 388

Trp Val Arg Val Ala Pro Gly Lys Gly Leu Glu Arg Ile Ser
1 5 10

<210> 389

<211> 17

<212> PRT

<213> -

<400> 389

Thr Cys Ile Thr Val Gly Asp Ser Thr Asn Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 390

<211> 16

<212> PRT

<213> -

<400> 390

Thr Ile Thr Val Gly Asp Ser Thr Ser Tyr Ala Asp Ser Val Lys Gly
1 5 10 15

<210> 391

<211> 17

<212> PRT

<213> -

<400> 391

Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 392

<211> 17

<212> PRT

<213> -

<400> 392

Ser Ile Asn Gly Arg Gly Asp Asp Thr Arg Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 393

<211> 17

<212> PRT

<213> -

<400> 393

Ala Ile Ser Ala Asp Ser Ser Thr Lys Asn Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 394

<211> 17

<212> PRT

<213> -

<400> 394

Ala Ile Ser Ala Asp Ser Ser Asp Lys Arg Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 395

<211> 18

<212> PRT

<213> -

<400> 395

Arg Asp Ile Ser Thr Gly Gly Gly Tyr Ser Tyr Tyr Ala Asp Ser Val
1 5 10 15

Lys Gly

<210> 396

<211> 32

<212> PRT

<213> -

<400> 396

Arg Phe Thr Ile Ser Met Asp Tyr Thr Lys Gln Thr Val Tyr Leu His
1 5 10 15

Met Asn Ser Leu Arg Pro Glu Asp Thr Gly Leu Tyr Tyr Cys Lys Ile
20 25 30

<210> 397

<211> 32

<212> PRT

<213> -

<400> 397

Arg Phe Thr Ile Ser Arg Asp Tyr Asp Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Arg Pro Glu Asp Thr Gly Leu Tyr Tyr Cys Lys Ile
20 25 30

<210> 398

<211> 32

<212> PRT

<213> -

<400> 398

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Thr Ile
20 25 30

<210> 399

<211> 32

<212> PRT

<213> -

<400> 399

Arg Phe Ser Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Glu Tyr Tyr Cys Thr Ile
20 25 30

<210> 400

<211> 32

<212> PRT

<213> -

<400> 400

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Lys Met Leu Tyr Leu Glu
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Val Ile
20 25 30

<210> 401

<211> 32

<212> PRT

<213> -

<400> 401

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Lys Met Leu Tyr Leu Glu
1 5 10 15

Met Asn Ser Leu Lys Ser Glu Asp Thr Ala Val Tyr Tyr Cys Val Ile
20 25 30

<210> 402

<211> 32

<212> PRT

<213> -

<400> 402

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Lys
20 25 30

<210> 403

<211> 8

<212> PRT

<213> -

<400> 403

Arg Arg Thr Trp His Ser Glu Leu
1 5

<210> 404

<211> 8

<212> PRT

<213> -

<400> 404

Arg Arg Thr Trp His Ser Glu Leu
1 5

<210> 405

<211> 6

<212> PRT

<213> -

<400> 405

Gly Gly Ser Leu Ser Arg
1 5

<210> 406

<211> 7

<212> PRT

<213> -

<400> 406

Gly Arg Ser Val Ser Arg Ser
1 5

<210> 407

<211> 5

<212> PRT

<213> -

<400> 407

Gly Arg Gly Ser Pro
1 5

<210> 408

<211> 5

<212> PRT

<213> -

<400> 408

Gly Arg Gly Ser Pro
1 5

<210> 409

<211> 13

<212> PRT

<213> -

<400> 409

Asp Arg Glu Ala Gln Val Asp Thr Leu Asp Phe Asp Tyr
1 5 10

<210> 410

<211> 11

<212> PRT

<213> -

<400> 410

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 411

<211> 11

<212> PRT

<213> -

<400> 411

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 412

<211> 11

<212> PRT

<213> -

<400> 412

Ser Ser Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 413

<211> 11

<212> PRT

<213> -

<400> 413

Arg Thr Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 414

<211> 11

<212> PRT

<213> -

<400> 414

Ser Ser Pro Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 415

<211> 11

<212> PRT

<213> -

<400> 415

Ala Ser Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 416

<211> 11

<212> PRT

<213> -

<400> 416

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 417

<211> 363

<212> PRT

<213> -

<400> 417

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln Leu
115 120 125

Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn Ser Leu Arg Leu
130 135 140

Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe Gly Met Ser Trp
145 150 155 160

Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ser Ile Ser
165 170 175

Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val Lys Gly Arg Phe
180 185 190

Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr Leu Gln Met Asn
195 200 205

Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys Thr Ile Gly Gly
210 215 220

Ser Leu Ser Arg Ser Ser Gln Gly Thr Leu Val Thr Val Ser Ser Gly
225 230 235 240

Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly
245 250 255

Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala
260 265 270

Ser Gly Phe Thr Phe Ser Asp Tyr Trp Met Tyr Trp Val Arg Gln Ala
275 280 285

Pro Gly Lys Gly Leu Glu Trp Val Ser Glu Ile Asn Thr Asn Gly Leu
290 295 300

Ile Thr Lys Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg
305 310 315 320

Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Pro
325 330 335

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Pro Ser Gly Phe Asn
340 345 350

Arg Gly Gln Gly Thr Leu Val Thr Val Ser Ser
355 360

<210> 418

<211> 379

<212> PRT

<213> -

<400> 418

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val
130 135 140

Gln Pro Gly Asn Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
145 150 155 160

Phe Ser Ser Phe Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly
165 170 175

Leu Glu Trp Val Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr
180 185 190

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
195 200 205

Thr Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala
210 215 220

Val Tyr Tyr Cys Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly
225 230 235 240

Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser
245 250 255

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
260 265 270

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Leu Ser Asn Tyr
275 280 285

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
290 295 300

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
305 310 315 320

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
325 330 335

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
340 345 350

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
355 360 365

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
370 375

<210> 419

<211> 260

<212> PRT

<213> -

<400> 419

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
145 150 155 160

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp
165 170 175

Tyr Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
180 185 190

Val Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser
195 200 205

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu
210 215 220

Tyr Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr
225 230 235 240

Cys Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val
245 250 255

Thr Val Ser Cys
260

<210> 420

<211> 264

<212> PRT

<213> -

<400> 420

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
145 150 155 160

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp
165 170 175

Tyr Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
180 185 190

Val Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser
195 200 205

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu
210 215 220

Tyr Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr
225 230 235 240

Cys Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val
245 250 255

Thr Val Ser Ser Gly Gly Gly Cys
260

<210> 421

<211> 20

<212> DNA

<213> -

<400> 421
ggataacaat ttcacacagg 20

<210> 422

<211> 55

<212> DNA

<213> -

<400> 422
tcagtaacct ggatccgcca ccgctgcctc caccgcctga ggagacggtg accag 55

<210> 423

<211> 39

<212> DNA

<213> -

<400> 423
aggttactga ggatccgagg tgcagctggt ggagtctgg 39

<210> 424

<211> 46

<212> DNA

<213> -

<400> 424
tcagtaacct tccggaaccg ccaccgcctg aggagacggt gacaag 46

<210> 425

<211> 48

<212> DNA

<213> -

<400> 425
aggttactga tccggaggcg gtagcgaggt gcagctggtg gagtctgg 48

<210> 426

<211> 19

<212> DNA

<213> -

<400> 426
cacgacgttg taaaacgac 19

<210> 427

<211> 43

<212> DNA

<213> -

<400> 427
atggtggtgt gcggccgcct attatgagga gacggtgacc agg 43

<210> 428

<211> 45

<212> DNA

<213> -

<400> 428
aggggtatct ctcgagaaaa gagaggtgca gctggtggag tctgg 45

<210> 429

<211> 56

<212> DNA

<213> -

<400> 429
gctaaagaag aaggggtatc tctcgagaaa agagaggtgc agctggtgga gtctgg 56

<210> 430

<211> 88

<212> DNA

<213> -

<400> 430
tcagtaacct ggatcccccg ccaccgctgc ctccaccgcc gctacccccg ccaccgctgc 60

ctccaccgcc tgaggagacg gtgacaag 88

<210> 431

<211> 69

<212> DNA

<213> -

<400> 431
aggttactga ggatccggcg gtggaggcag cggtggcggg ggtagcgagg tgcagctggt 60

ggagtctgg 69

<210> 432

<211> 41

<212> DNA

<213> -

<400> 432
atggtggtgt gaattcttat tagcaggaga cggtgacaag g 41

<210> 433

<211> 53

<212> DNA

<213> -

<400> 433
atggtggtgt gaattcttat tagcaacctc cacctgagga gacggtgaca agg 53

<210> 434

<211> 21

<212> DNA

<213> -

<400> 434
gactggttcc aattgacaag c 21

<210> 435

<211> 21

<212> DNA

<213> -

<400> 435
gcaaatggca ttctgacatc c 21

CLAIMS

1. Nanobody against TNF-alpha, which consists of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), in which:

d) CDR1 comprises:

- the amino acid sequence DYWMY; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence DYWMY; or
- an amino acid sequences that has only 1 amino acid difference with the amino acid sequence DYWMY;

and

e) CDR2 comprises:

- the amino acid sequence EINTNGLITKYPDSVKG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence EINTNGLITKYPDSVKG; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence EINTNGLITKYPDSVKG;

and

f) CDR3 comprises:

- the amino acid sequence SPSGFN; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence SPSGFN; or
- an amino acid sequences that has only 1 amino acid difference with the amino acid sequence SPSGFN.

2. Nanobody according to claim 1, in which CDR1 comprises the amino acid sequence DYWMY.

3. Nanobody according to claim 1, in which CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG.

4. Nanobody according to claim 1, in which CDR3 comprises the amino acid sequence SPSGFN.

5. Nanobody according to claim 1, in which:

- CDR1 comprises the amino acid sequence DYWMY; and CDR3 comprises the amino acid sequence SPSGFN; or
- CDR1 comprises the amino acid sequence DYWMY; and CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG; or
- CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG; and CDR3 comprises the amino acid sequence SPSGFN

6. Nanobody according to claim 1, in which CDR1 comprises the amino acid sequence DYWMY; and CDR3 comprises the amino acid sequence SPSGFN.

7. Nanobody according to claim 1, in which CDR1 comprises the amino acid sequence DYWMY; CDR2 comprises the amino acid sequence EINTNGLITKYPDSVKG and CDR3 comprises the amino acid sequence SPSGFN.

8. Nanobody according to any of claims 1-7, in which

- any amino acid substitution is a conservative amino acid substitution; and/or
- said amino acid sequence only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s).

9. Nanobody according to any of claims 1-8, which is a humanized Nanobody.

10. Nanobody according to any of claims 1-9, which is a GLEW-class Nanobody.

11. Nanobody according to any of claims 1-9, which contains an arginine residue (R) at position 103.

12. Nanobody according to any of claims 1-9, which is a GLEW-class Nanobody.

13. Nanobody according to any of claims 1-9, which is a GLEW-class Nanobody with an arginine residue (R) at position 103.

14. Nanobody according to any of claims 1-13, which has at least 80%, or at least 90%, or at least 95%, or at least 99% sequence identity (as defined herein) with one of the amino acid sequences of SEQ ID NO's 52 (TNF1), 76 (TNF13), 77 (TNF14), 95 (TNF29) or 96 (TNF30).

15. Nanobody according to any of claims 1-14, which contains a leucine residue (L) at position 108.

475.

16. Nanobody according to any of claims 1-8, which has a K_{off} rate for TNF of better than 2.10-3 (1/s), preferably better than 1.10-3 (1/s); or a humanized variant of such a Nanobody.

17. Nanobody according to any of claims 1-8, which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than the EC50 value of Nanobody VHH 3E (SEQ ID NO:4) of WO 04/041862 in the same assay; or a humanized variant of such a Nanobody.

18. Nanobody according to any of claims 1-8, which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 5nM; or a humanized variant of such a Nanobody.

19. Nanobody according to any of claims 1-8, which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 3nM; or a humanized variant of such a Nanobody.

20. Nanobody against TNF-alpha, which consists of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), in which

d) CDR1 is:

- the amino acid sequence DYWMY; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence DYWMY; or
- an amino acid sequences that has only 1 amino acid difference with the amino acid sequence DYWMY;

and in which:

e) CDR2 is:

- the amino acid sequence EINTNGLITKYPDSVKG; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence EINTNGLITKYPDSVKG; or
- an amino acid sequences that has 2 or only 1 amino acid difference(s) with the amino acid sequence EINTNGLITKYPDSVKG;

and in which

f) CDR3 is:

- the amino acid sequence SPSGFN; or
- an amino acid sequence that has at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the amino acid sequence SPSGFN; or
- an amino acid sequences that has only 1 amino acid difference with the amino acid sequence SPSGFN.

21. Nanobody according to claim 20, in which CDR1 is the amino acid sequence DYWMY.

22. Nanobody according to claim 20, in which CDR2 is the amino acid sequence EINTNGLITKYPDSVKG.

23. Nanobody according to claim 20, in which CDR3 is the amino acid sequence SPSGFN

24. Nanobody according to claim 20, in which:
- CDR1 is the amino acid sequence DYWMY; and CDR3 is the amino acid sequence SPSGFN; or
- CDR1 is the amino acid sequence DYWMY; and CDR2 is the amino acid sequence EINTNGLITKYPDSVKG; or
- CDR2 is the amino acid sequence EINTNGLITKYPDSVKG; and CDR3 is the amino acid sequence SPSGFN

25. Nanobody according to claim 20, in which CDR1 is the amino acid sequence DYWMY; and CDR3 is the amino acid sequence SPSGFN.

26. Nanobody according to claim 20, in which CDR1 is the amino acid sequence DYWMY; CDR2 is the amino acid sequence EINTNGLITKYPDSVKG and CDR3 is the amino acid sequence SPSGFN.

27. A Nanobody according to any of claims 20-26, in which:
- any amino acid substitution is preferably a conservative amino acid substitution; and/or
- said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s).

28. Nanobody according to any of claims 20-27, which is a humanized Nanobody.

29. Nanobody according to any of claims 20-27, which is a GLEW-class Nanobody.

30. A Nanobody according to any of claims 20-27, which contains an arginine residue (R) at position 103.

31. Nanobody according to any of claims 20-27, Nanobody according to any of claims 1-8, which is a GLEW-class Nanobody with an arginine residue (R) at position 103.

32. Nanobody according to any of claims 20-27, which has at least 80%, or at least 90%, or at least 95%, or at least 99% sequence identity (as defined herein) with one of the amino acid sequences of SEQ ID NO's 52 (TNF1), 76 (TNF13), 77 (TNF14), 95 (TNF29) or 96 (TNF30).

33. Nanobody according to any of claims 20-27, which contains a leucine residue (L) at position 108.

34. Nanobody according to any of claims 20-27, which has a K_{off} rate for TNF of better than 2.10-3 (1/s), preferably better than 1.10-3 (1/s); or a humanized variant of such a Nanobody.

35. Nanobody according to any of claims 20-27, which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than the EC50 value of Nanobody VHH 3E (SEQ ID NO:4) of WO 04/041862 in the same assay or a humanized variant of such a Nanobody.

36. Nanobody according to any of claims 20-27, which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 5nM; or a humanized variant of such a Nanobody.

37. Nanobody according to any of claims 20-27, which has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than 3nM; or a humanized variant of such a Nanobody.

38. Nanobody according to any of claims 1-37, which is chosen from the group consisting of TNF 13 (SEQ ID NO: 76), TNF 14 (SEQ ID NO: 77), TNF 29 (SEQ ID NO: 95) and TNF 30 (SEQ ID NO:96).

39. Nanobody according to any of claims 1-38, which is TNF 30 (SEQ ID NO: 96).

40. Protein or polypeptide, which comprises or essentially consists of a Nanobody according to any of claims 1-39.

41. Protein or polypeptide according to claim 40, which comprises or essentially consists of at least one Nanobody according to any of claims 1-39.

42. Protein or polypeptide according to any of claims 40-41, which comprises two Nanobodies according to any of claims 1-39.

43. Protein or polypeptide according to any of claims 40-42, which comprises two Nanobodies according to any of claims 1-39, and which is such that said protein or

polypeptide, upon binding to a TNF trimer, is capable inhibiting or reducing the TNF receptor crosslinking that is mediated by said TNF trimer and/or the signal transduction that is mediated by such receptor crosslinking.

44. Protein or polypeptide according to any of claims 40-42, which comprises two Nanobodies according to any of claims 1-39, and which is capable of intramolecular binding to at least two TNF receptor binding sites on a TNF trimer.

45. Protein or polypeptide according to any of claims 42-44, which comprises two Nanobodies according to any of claims 1-39, which are directly linked to each other or linked to each other via a linker.

46. Protein or polypeptide according to claim 45, in which the linker is an amino acid sequence.

47. Protein or polypeptide according to claim 46, in which the linker is an amino acid sequence that comprises at least 14 amino acids, more preferably at least 17 amino acids, such as about 20-40 amino acids.

48. Protein or polypeptide according to claim 47, in which the linker comprises glycine and serine residues.

49. Protein or polypeptide according to any of claims 42-48, which comprises or essentially consists of the polypeptide TNF 7 (SEQ ID NO: 73), in which both Nanobodies TNF 1 have been humanized.

50. Protein or polypeptide according to any of claims 42-49, which comprises or essentially consists the polypeptide TNF 55 (SEQ ID NO: 419) or TNF 56 (SEQ ID NO: 420).

51. Protein or polypeptide according to any of claims 40-49, which is pegylated.

52. Protein or polypeptide according to any of claims 40-49, which further comprises at least one Nanobody directed against human serum albumin.

53. Protein or polypeptide according to claim 52, wherein the at least one Nanobody directed against human serum albumin has the CDR sequences present in ALB 8 (SEQ ID NO: 102).

54. Protein or polypeptide according to claim 42, which comprises two Nanobodies according to any of claims 1-39, which further comprises at least one Nanobody directed against human serum albumin.

55. Protein or polypeptide according to claim 54, and in which the two Nanobodies according to any of claims 1-39 are either linked directly to the at least one Nanobody

directed against human serum albumin, or are linked to the at least one Nanobody directed against human serum albumin via a linker.

56. Protein or polypeptide according to claim 55, in which the linker is an amino acid sequence.

57. Protein or polypeptide according to claim 55, in which the linker is an amino acid sequence that comprises between 3 and 40 amino acid residues, such as between 5 and 15 amino acid residues

58. Protein or polypeptide according to any of claims 56 and 57, in which the linker consists of glycine and serine residues.

59. Protein or polypeptide according to claim 54-58, wherein the at least one Nanobody directed against human serum albumin has the CDR sequences present in ALB 8 (SEQ ID NO: 102).

60. Protein or polypeptide according to any of claims 54-59, in which the at least one Nanobody directed against human serum albumin is a humanized Nanobody.

61. Protein or polypeptide according to claim 60, in which the at least one Nanobody directed against human serum albumin is a humanized variant of the Nanobody ALB 1 (SEQ ID NO: 63).

62. Protein or polypeptide according to claim 60, in which the at least one Nanobody directed against human serum albumin is a chosen from the group consisting of ALB 3 (SEQ ID NO: 87), ALB 4 (SEQ ID NO: 88), ALB 5 (SEQ ID NO: 89), ALB 6 (SEQ ID NO: 100), ALB 7 (SEQ ID NO: 101), ALB 8 (SEQ ID NO: 102), ALB 9 (SEQ ID NO: 103) and ALB 10 (SEQ ID NO: 104).

63. Protein or polypeptide according to claim 62, in which the at least one Nanobody directed against human serum albumin is ALB 8 (SEQ ID NO: 102).

64. Protein or polypeptide according to any of claims 54-63, which comprises or essentially consists of two Nanobodies which are humanized variants of a Nanobody according to any of claims 1-39 and one humanized variant of the Nanobody ALB 1 (SEQ ID NO: 63).

65. Protein or polypeptide according to any of claims 54-64, which comprises or essentially consists of the polypeptide TNF24 (SEQ ID NO: 90), in which both the Nanobody TNF 1 as well as the Nanobody ALB 1 has been humanized.

66. Protein or polypeptide according to any of claims 54-65, which comprises or essentially consists of two Nanobodies TNF 30 and one Nanobody ALB 8.

67. Protein or polypeptide according to any of claims 54-66, which comprises or essentially consists the polypeptide TNF 60 (SEQ ID NO: 417).

68. Nanobody against TNF-alpha, which is a humanized variant of a Nanobody against TNF-alpha, which Nanobody against TNF-alpha has the following framework sequences: FR1: SEQ ID NO: 130; FR2: SEQ ID NO: 198; FR3: SEQ ID NO: 266; and FR4: SEQ ID NO: 334.

69. Nanobody according to claim 68, which has CDR's that are such that the Nanobody has a Koff rate for TNF of better than 2.10-3 (1/s), preferably better than 1.10-3 (1/s).

70. Nanobody according to claim 68 or 69, which has CDR's that are such that the Nanobody has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than the EC50 value of Nanobody VHH 3E (SEQ ID NO:4) of WO 04/041862 in the same assay; and in particular better than 12nM, more in particular better than 5 nM, even more in particular better than 3nM.

71. Nanobody according to any of claims 68-70, which is directed against the same epitope of TNF (i.e. the TNF trimer) as TNF1.

72. Nanobody which is directed against the same epitope on the trimer of TNF-alpha as Nanobody TNF1 (SEQ ID NO: 52).

73. A Nanobody which is directed against the same epitope on the trimer of TNF-alpha as Nanobody TNF3 (SEQ ID NO: 60).

74. Nanobody according to claim 73, which has CDR's that are such that the Nanobody has a Koff rate for TNF of better than 2.10-3 (1/s), preferably better than 1.10-3 (1/s).

75. Nanobody according to claim 73 or claim 74, which has CDR's that are such that the Nanobody has an EC50 value in the cell-based assay using KYM cells described in Example 1, under 3), of WO 04/041862 that is better than the EC50 value of Nanobody VHH 3E (SEQ ID NO:4) of WO 04/041862 in the same assay; and in particular better than 12nM, more in particular better than 5 nM, even more in particular better than 3nM.

76. Nanobody, which is a humanized variant of the Nanobody TNF1 (SEQ ID NO: 52).

77. Nanobody according to claim 76, which has CDR's that are such that the Nanobody has a Koff rate for TNF of better than 2.10-3 (1/s), preferably better than 1.10-3 (1/s).

78. Nanobody according to claim 76 or claims 77, which has CDR's that are such that the Nanobody has an EC50 value in the cell-based assay using KYM cells described in

Example 1, under 3), of WO 04/041862 that is better than the EC50 value of Nanobody VHH 3E (SEQ ID NO:4) of WO 04/041862 in the same assay; and in particular better than 12nM, more in particular better than 5 nM, even more in particular better than 3nM.

79. Protein or polypeptide, which comprises or essentially consists of a Nanobody according to any of claims 68-78.

80. Protein or polypeptide according to claim 79, which comprises or essentially consists of at least one Nanobody according to any of claims 68-78.

81. Protein or polypeptide according to any of claims 79-80, which comprises two Nanobodies according to any of claims 68-78.

82. Protein or polypeptide according to any of claims 79-81, which comprises two Nanobodies according to any of claims 68-78, and which is such that said protein or polypeptide, upon binding to a TNF trimer, is capable inhibiting or reducing the TNF receptor crosslinking that is mediated by said TNF trimer and/or the signal transduction that is mediated by such receptor crosslinking.

83. Protein or polypeptide according to any of claims 79-81, which comprises two Nanobodies according to any of claims 68-78, and which is capable of intramolecular binding to at least two TNF receptor binding sites on a TNF trimer.

84. Protein or polypeptide according to any of claims 81-83, which comprises two Nanobodies according to any of claims 68-78, which are directly linked to each other or linked to each other via a linker.

85. Protein or polypeptide according to claim 84, in which the linker is an amino acid sequence.

86. Protein or polypeptide according to claim 85, in which the linker is an amino acid sequence that comprises at least 14 amino acids, more preferably at least 17 amino acids, such as about 20-40 amino acids.

87. Protein or polypeptide according to claim 86, in which the linker comprises glycine and serine residues.

88. Protein or polypeptide according to any of claims 81-83, which comprises or essentially consists of the polypeptide TNF 7 (SEQ ID NO: 73), in which both Nanobodies TNF 1 have been humanized.

89. Protein or polypeptide according to any of claims 80-88, which is pegylated.

90. Protein or polypeptide according to claim 80, which comprises two Nanobodies according to any of claims 68-78, and which protein or polypeptide further comprises at least one Nanobody directed against human serum albumin.

91. Protein or polypeptide according to claim 90, and in which the two Nanobodies according to any of claims 68-78 are either linked directly to the at least one Nanobody directed against human serum albumin, or are linked to the at least one Nanobody directed against human serum albumin via a linker.

92. Protein or polypeptide according to claim 91, in which the linker is an amino acid sequence.

93. Protein or polypeptide according to claim 92, in which the linker is an amino acid sequence that comprises between 3 and 40 amino acid residues, such as between 5 and 15 amino acid residues

94. Protein or polypeptide according to any of claims 92 or 93, in which the linker consists of glycine and serine residues.

95. Protein or polypeptide according to claim any of claims 90-94, wherein the at least one Nanobody directed against human serum albumin has the CDR sequences present in ALB 8 (SEQ ID NO: 102).

96. Protein or polypeptide according to any of claims 90-95, in which the at least one Nanobody directed against human serum albumin is a humanized Nanobody.

97. Protein or polypeptide according to claim 96, in which the at least one Nanobody directed against human serum albumin is a humanized variant of the Nanobody ALB 1 (SEQ ID NO: 63).

98. Protein or polypeptide according to claim 97, in which the at least one Nanobody directed against human serum albumin is a chosen from the group consisting of ALB 3 (SEQ ID NO: 87), ALB 4 (SEQ ID NO: 88), ALB 5 (SEQ ID NO: 89), ALB 6 (SEQ ID NO: 100), ALB 7 (SEQ ID NO: 101), ALB 8 (SEQ ID NO: 102), ALB 9 (SEQ ID NO: 103) and ALB 10 (SEQ ID NO: 104).

99. Protein or polypeptide according to claim 98, in which the at least one Nanobody directed against human serum albumin is ALB 8 (SEQ ID NO: 102).

100. Protein or polypeptide according to any of claims 81-99, which comprises or essentially consists of two Nanobodies which are humanized variants of a Nanobody according to any of claims 65-67 and one humanized variant of the Nanobody ALB 1 (SEQ ID NO: 63).

101. A protein or polypeptide that comprises or essentially consists of two or more immunoglobulin variable domains (or suitable fragments thereof) that are each directed against epitopes on TNF-alpha (and in particular of the TNF-alpha trimer) that lie in and/or form part of the receptor binding site(s) of the TNF trimer, such that said polypeptide, upon binding to a TNF trimer, is capable of inhibiting or reducing the TNF receptor crosslinking that is mediated by said TNF trimer and/or the signal transduction that is mediated by such receptor crosslinking..

102. A protein or polypeptide that comprises or essentially consists of two or more immunoglobulin variable domains (or suitable fragments thereof) that are each directed against epitopes on TNF-alpha (and in particular of the TNF-alpha trimer) that lie in and/or form part of the receptor binding site(s) of the TNF trimer, such that said polypeptide is capable of intramolecular binding to at least two TNF receptor binding sites on a TNF trimer.

103. Protein or polypeptide according to claim 101 or 102, in which the two or more immunoglobulin variable domains are directed against the same epitope as Nanobody TNF1 (SEQ ID NO: 52).

104. Protein or polypeptide according to claim 101 or 102, in which the two or more immunoglobulin variable domains are Nanobodies.

105. Nucleotide sequence or nucleic acid, encoding a Nanobody, protein or polypeptide according to any of the preceding claims.

106. Host cell, comprising a nucleotide sequence or nucleic acid according to claim 105, or which expresses or is capable of expressing a Nanobody, protein or polypeptide according to any of claims 1-104.

107. Method for preparing a Nanobody, protein or polypeptide according to any of claims 1-102, which comprises cultivating or maintaining a host cell according to claim 106 under conditions such that said host cell produces or expresses a Nanobody, protein or polypeptide according to any of claims 1-104; and which optionally further comprises isolating the Nanobody, protein or polypeptide according to any of claims 1-104.

108. Pharmaceutical composition, comprising at least one Nanobody, protein or polypeptide according to any of claims 1-104, and optionally at least one pharmaceutically acceptable carrier.

109. A pharmaceutical composition according to claim 108, or a Nanobody, protein or polypeptide according to any of claims 1-104, for the treatment of at least one disease

and disorder chosen from the group consisting of inflammation, rheumatoid arthritis, COPD, asthma, Crohn's disease, ulcerative colitis, inflammatory bowel syndrome, multiple sclerosis, Addison's disease, Autoimmune hepatitis, Autoimmune parotitis, Diabetes Type I, Epididymitis, Glomerulonephritis, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, Hemolytic anemia, Systemic lupus erythematosus, Male infertility, Multiple sclerosis, Myasthenia Gravis, Pemphigus, Psoriasis, Rheumatic fever, Rheumatoid arthritis, Sarcoidosis, Scleroderma, Sjogren's syndrome, Spondyloarthropathies, Thyroiditis, and Vasculitis.

110. Use of a Nanobody, protein or polypeptide according to any of claims 1-104, in the preparation of a medicament for the treatment of at least one disease and disorder chosen from the group consisting of inflammation, rheumatoid arthritis, COPD, asthma, Crohn's disease, ulcerative colitis, inflammatory bowel syndrome, multiple sclerosis, Addison's disease, Autoimmune hepatitis, Autoimmune parotitis, Diabetes Type I, Epididymitis, Glomerulonephritis, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, Hemolytic anemia, Systemic lupus erythematosus, Male infertility, Multiple sclerosis, Myasthenia Gravis, Pemphigus, Psoriasis, Rheumatic fever, Rheumatoid arthritis, Sarcoidosis, Scleroderma, Sjogren's syndrome, Spondyloarthropathies, Thyroiditis, and Vasculitis.

111. Protein or polypeptide, which has at least 80%, or at least 90%, or at least 95%, or at least 99% sequence identity (as defined herein) with the polypeptide TNF 60 (SEQ ID NO: 417)

ABSTRACT

The present invention relates to improved Nanobodies™ against Tumor Necrosis Factor-alpha (TNF-alpha), as well as to polypeptides comprising or essentially consisting of one or more of such Nanobodies. The invention also relates to nucleic acids encoding such Nanobodies and polypeptides; to methods for preparing such Nanobodies and polypeptides; to host cells expressing or capable of expressing such Nanobodies or polypeptides; to compositions comprising such Nanobodies, polypeptides, nucleic acids or host cells; and to uses of such Nanobodies, such polypeptides, such nucleic acids, such host cells or such compositions, in particular for prophylactic, therapeutic or diagnostic purposes, such as the prophylactic, therapeutic or diagnostic purposes.

amended sequence listing1

SEQUENCE LISTING

<110> Ablynx N.V.

<120> Improved Nanobodies™ against Tumor Necrosis Factor-alpha

<130> ABL-027-PCT

<140> PCT/EP2006/004678
<141> 2006-05-17

<150> 60/682,332
<151> 2005-05-18

<160> 476

<170> PatentIn version 3.3

<210> 1
<211> 26
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<220>
<221> MISC_FEATURE
<222> (11)..(11)
<223> Hallmark residue

<400> 1

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Xaa Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
20 25

<210> 2
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<220>
<221> MISC_FEATURE
<222> (2)..(2)
<223> Hallmark residue

<220>
<221> MISC_FEATURE
<222> (9)..(10)
<223> Hallmark residue

<220>
<221> MISC_FEATURE

<222> (12)..(12)
<223> Hallmark residue

<400> 2

Trp Xaa Arg Gln Ala Pro Gly Lys Xaa Xaa Glu Xaa Val Ala
1 5 10

<210> 3
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<220>
<221> MISC_FEATURE
<222> (21)..(22)
<223> Hallmark residue

<400> 3

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Xaa Xaa Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 4
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<220>
<221> MISC_FEATURE
<222> (1)..(2)
<223> Hallmark residue

<220>
<221> MISC_FEATURE
<222> (6)..(6)
<223> Hallmark residue

<400> 4

Xaa Xaa Gln Gly Thr Xaa Val Thr Val Ser Ser
1 5 10

<210> 5
<211> 26
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 5

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
20 25

<210> 6
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 6

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Val Ala
1 5 10

<210> 7
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 7

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 8
<211> 13
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 8

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Ala
1 5 10

<210> 9
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 9

Trp Phe Arg Gln Ala Pro Gly Lys Gln Arg Glu Leu Val Ala
1 5 10

<210> 10
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 10

Trp Phe Arg Gln Ala Pro Gly Lys Gln Arg Glu Phe Val Ala
1 5 10

<210> 11
<211> 13
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 11

Trp Tyr Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ala
1 5 10

<210> 12
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 12

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 13
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 13

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser

1 5 10

<210> 14
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 14

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> 15
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 15

Asp Tyr Trp Met Tyr
1 5

<210> 16
<211> 10
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 16

Glu Pro Ser Gly Tyr Thr Tyr Thr Ile Gly
1 5 10

<210> 17
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 17

Asn Tyr Tyr Met Gly
1 5

<210> 18
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 18

Val Ser Trp Met Tyr
1 5

<210> 19
<211> 8
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 19

Ala His Ser Val Tyr Thr Met Gly
1 5

<210> 20
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 20

Gly Tyr Tyr Met Gly
1 5

<210> 21
<211> 10
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 21

Asp Tyr Ser Gly Tyr Thr Tyr Thr Val Gly
1 5 10

<210> 22
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 22

Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val Lys

1 5 10 15

Gly

<210> 23
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 23

Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 24
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 24

Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val Lys
1 5 10 15

Gly

<210> 25
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 25

Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Val Asp Ser Val Lys
1 5 10 15

Gly

<210> 26
<211> 17

<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 26

Arg Ile Tyr Trp Ser Ser Gly Asn Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 27
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 27

Arg Ile Tyr Trp Ser Ser Ala Asn Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 28
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 28

Ser Ile Ser Trp Arg Gly Asp Asn Thr Tyr Tyr Lys Glu Ser Val Lys
1 5 10 15

Gly

<210> 29
<211> 15
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 29

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Gly Ser Tyr Asn Tyr

1 5 10 15

<210> 30
<211> 14
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 30

Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
1 5 10

<210> 31
<211> 15
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 31

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Glu Ala Tyr Asn Tyr
1 5 10 15

<210> 32
<211> 14
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 32

Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Asn
1 5 10

<210> 33
<211> 15
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 33

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Glu Ser Tyr Asn Tyr
1 5 10 15

<210> 34
<211> 6
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 34

Ser Pro Ser Gly Phe Asn
1 5

<210> 35
<211> 6
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 35

Ser Pro Ser Gly Ser Phe
1 5

<210> 36
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 36

Ser Phe Gly Met Ser
1 5

<210> 37
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 37

Leu Asn Leu Met Gly
1 5

<210> 38
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 38

Ile Asn Leu Leu Gly

1 5

<210> 39
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 39

Asn Tyr Trp Met Tyr
1 5

<210> 40
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 40

Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 41
<211> 16
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 41

Thr Ile Thr Val Gly Asp Ser Thr Asn Tyr Ala Asp Ser Val Lys Gly
1 5 10 15

<210> 42
<211> 16
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 42

Thr Ile Thr Val Gly Asp Ser Thr Ser Tyr Ala Asp Ser Val Lys Gly
1 5 10 15

<210> 43
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 43

Ser Ile Asn Gly Arg Gly Asp Asp Thr Arg Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 44
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 44

Ala Ile Ser Ala Asp Ser Ser Thr Lys Asn Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 45
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 45

Ala Ile Ser Ala Asp Ser Ser Asp Lys Arg Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 46
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 46

Arg Ile Ser Thr Gly Gly Gly Tyr Ser Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 47
<211> 13
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 47

Asp Arg Glu Ala Gln Val Asp Thr Leu Asp Phe Asp Tyr
1 5 10

<210> 48
<211> 6
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 48

Gly Gly Ser Leu Ser Arg
1 5

<210> 49
<211> 8
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 49

Arg Arg Thr Trp His Ser Glu Leu
1 5

<210> 50
<211> 7
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 50

Gly Arg Ser Val Ser Arg Ser
1 5

<210> 51
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 51

Gly Arg Gly Ser Pro
1 5

<210> 52
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 52

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 53
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 53

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Met Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Gly Val Ser
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Ser Phe Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 54
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 54

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Asp Phe Ser Val Ser
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Val Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asp Ser Leu Ile Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Ser Phe Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 55
<211> 129
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 55

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Glu Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Leu Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 56

<211> 129
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 56

Gln Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Asp Tyr
20 25 30

Ser Gly Tyr Thr Tyr Thr Val Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Glu Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Asn Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Leu Met Asn Asn Leu Glu Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Glu Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 57
<211> 129
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 57

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Asp Tyr
20 25 30

Ser Gly Tyr Thr Tyr Thr Val Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Glu Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Asn Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Leu Met Asn Asn Leu Glu Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Glu Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 58
<211> 127
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 58

Ala Val Gln Leu Val Asp Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Ala His
20 25 30

Ser Val Tyr Thr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg
35 40 45

Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Ala Asn Thr Tyr Tyr Ala
50 55 60

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
65 70 75 80

Thr Val Asp Leu Leu Met Asn Cys Leu Lys Pro Glu Asp Thr Ala Val
85 90 95

Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Glu

100 105 110

Ala Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120 125

<210> 59
<211> 123
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 59

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ala Ala Ser Gly Arg Ser Phe Thr Gly Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Gln Leu Leu
35 40 45

Ala Ser Ile Ser Trp Arg Gly Asp Asn Thr Tyr Tyr Lys Glu Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Asn
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 60
<211> 123
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 60

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 61
<211> 117
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 61

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Gly Gly Gly
1 5 10 15

Ser Leu Arg Leu Ala Cys Ala Ala Ser Glu Arg Ile Phe Asp Leu Asn
20 25 30

Leu Met Gly Trp Tyr Arg Gln Gly Pro Gly Asn Glu Arg Glu Leu Val
35 40 45

Ala Thr Cys Ile Thr Val Gly Asp Ser Thr Asn Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Met Asp Tyr Thr Lys Gln Thr Val Tyr
65 70 75 80

Leu His Met Asn Ser Leu Arg Pro Glu Asp Thr Gly Leu Tyr Tyr Cys
85 90 95

Lys Ile Arg Arg Thr Trp His Ser Glu Leu Trp Gly Gln Gly Thr Gln

100 105 110

Val Thr Val Ser Ser
115

<210> 62
<211> 116
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 62

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Glu Gly Gly
1 5 10 15

Ser Leu Arg Leu Ala Cys Ala Ala Ser Glu Arg Ile Trp Asp Ile Asn
20 25 30

Leu Leu Gly Trp Tyr Arg Gln Gly Pro Gly Asn Glu Arg Glu Leu Val
35 40 45

Ala Thr Ile Thr Val Gly Asp Ser Thr Ser Tyr Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Tyr Asp Lys Asn Thr Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Gly Leu Tyr Tyr Cys Lys
85 90 95

Ile Arg Arg Thr Trp His Ser Glu Leu Trp Gly Gln Gly Thr Gln Val
100 105 110

Thr Val Ser Ser
115

<210> 63
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 63

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 64
<211> 116
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 64

Ala Val Gln Leu Val Asp Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Phe Gly Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Tyr Pro Gly Lys Glu Pro Glu Trp Val
35 40 45

Ser Ser Ile Asn Gly Arg Gly Asp Asp Thr Arg Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Ser Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Glu Tyr Tyr Cys
85 90 95

Thr Ile Gly Arg Ser Val Ser Arg Ser Arg Thr Gln Gly Thr Gln Val

100 105 110

Thr Val Ser Ser
115

<210> 65
<211> 114
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 65

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Thr Cys Thr Ala Ser Gly Phe Thr Phe Arg Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Asp Gln Glu Trp Val
35 40 45

Ser Ala Ile Ser Ala Asp Ser Ser Thr Lys Asn Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Lys Met Leu Tyr
65 70 75 80

Leu Glu Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Val Ile Gly Arg Gly Ser Pro Ser Ser Pro Gly Thr Gln Val Thr Val
100 105 110

Ser Ser

<210> 66
<211> 114
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 66

Gln Val Gln Leu Ala Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Thr Cys Thr Ala Ser Gly Phe Thr Phe Gly Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Glu Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Ala Asp Ser Ser Asp Lys Arg Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Lys Met Leu Tyr
65 70 75 80

Leu Glu Met Asn Ser Leu Lys Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Val Ile Gly Arg Gly Ser Pro Ala Ser Gln Gly Thr Gln Val Thr Val
100 105 110

Ser Ser

<210> 67
<211> 123
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 67

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30

Trp Met Tyr Trp Val Arg Val Ala Pro Gly Lys Gly Leu Glu Arg Ile
35 40 45

Ser Arg Asp Ile Ser Thr Gly Gly Gly Tyr Ser Tyr Tyr Ala Asp Ser
50 55 60

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu
65 70 75 80

Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr
85 90 95

Cys Ala Lys Asp Arg Glu Ala Gln Val Asp Thr Leu Asp Phe Asp Tyr

100 105 110

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 68
<211> 9
<212> PRT
<213> Artificial

<220>
<223> linker

<400> 68

Gly Gly Gly Gly Ser Gly Gly Gly Ser
1 5

<210> 69
<211> 30
<212> PRT
<213> Artificial

<220>
<223> linker

<400> 69

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
20 25 30

<210> 70
<211> 239
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 70

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Gln Val Gln Leu
115 120 125

Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu
130 135 140

Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr Trp Met Tyr Trp
145 150 155 160

Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Glu Ile Asn
165 170 175

Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val Lys Gly Arg Phe
180 185 190

Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met Asn
195 200 205

Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg Ser Pro
210 215 220

Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
225 230 235

<210> 71
<211> 267
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 71

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

```
Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
        35                  40                  45

Glu Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
    50                  55                  60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65                  70                  75                  80

Lys Asn Thr Val Asp Leu Leu Met Asn Ser Leu Lys Pro Glu Asp Thr
                85                  90                  95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
            100                 105                 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
        115                 120                 125

Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Gln Val Gln Leu Val Glu
    130                 135                 140

Ser Gly Gly Gly Leu Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys
145                 150                 155                 160

Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro Ser Gly Tyr Thr Tyr Thr
                165                 170                 175

Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
            180                 185                 190

Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr Tyr Ala Asp Ser Val Lys
        195                 200                 205

Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala Lys Asn Thr Val Asp Leu
    210                 215                 220

Leu Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala
225                 230                 235                 240

Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Gly Ser Tyr Asn Tyr
                245                 250                 255

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
            260                 265
```

<210> 72
<211> 255

<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 72

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val
130 135 140

Gln Ala Gly Gly Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser
145 150 155 160

Leu Ser Asn Tyr Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu
165 170 175

Arg Glu Leu Leu Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr
180 185 190

Lys Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys
195 200 205

Asn Thr Ile Tyr Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala
210 215 220

Val Tyr Tyr Cys Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly
225 230 235 240

Trp Asn Thr Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
245 250 255

<210> 73
<211> 260
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 73

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
145 150 155 160

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp
165 170 175

Tyr Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
180 185 190

Val Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser
195 200 205

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu
210 215 220

Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr
225 230 235 240

Cys Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val
245 250 255

Thr Val Ser Ser
260

<210> 74
<211> 288
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 74

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Glu Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Leu Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser

115 120 125

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
130 135 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln
145 150 155 160

Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly Ser
165 170 175

Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro Ser
180 185 190

Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu
195 200 205

Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr Tyr
210 215 220

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala Lys
225 230 235 240

Asn Thr Val Asp Leu Leu Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
245 250 255

Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser Val
260 265 270

Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
275 280 285

<210> 75
<211> 276
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 75

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
130 135 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser
145 150 155 160

Gly Gly Gly Leu Val Gln Ala Gly Gly Ser Leu Ser Leu Ser Cys Ser
165 170 175

Ala Ser Gly Arg Ser Leu Ser Asn Tyr Tyr Met Gly Trp Phe Arg Gln
180 185 190

Ala Pro Gly Lys Glu Arg Glu Leu Leu Gly Asn Ile Ser Trp Arg Gly
195 200 205

Tyr Asn Ile Tyr Tyr Lys Asp Ser Val Lys Gly Arg Phe Thr Ile Ser
210 215 220

Arg Asp Asp Ala Lys Asn Thr Ile Tyr Leu Gln Met Asn Arg Leu Lys
225 230 235 240

Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala Ser Ile Leu Pro Leu
245 250 255

Ser Asp Asp Pro Gly Trp Asn Thr Tyr Trp Gly Gln Gly Thr Gln Val
260 265 270

Thr Val Ser Ser
275

<210> 76

<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 76

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 77
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 77

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 78
<211> 129
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 78

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 79
<211> 129
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 79

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 80
<211> 129
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 80

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 81
<211> 129
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 81

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr

85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 82
<211> 129
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 82

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
115 120 125

Ser

<210> 83
<211> 123

<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 83

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 84
<211> 123
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 84

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val

50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 85
<211> 123
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 85

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 86
<211> 123

<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 86

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 87
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 87

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val

50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 88
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 88

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 89
<211> 115

<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 89

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 90
<211> 363
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 90

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val

50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln Leu
115 120 125

Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn Ser Leu Arg Leu
130 135 140

Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe Gly Met Ser Trp
145 150 155 160

Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val Ser Ser Ile Ser
165 170 175

Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val Lys Gly Arg Phe
180 185 190

Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr Leu Gln Met Asn
195 200 205

Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Thr Ile Gly Gly
210 215 220

Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr Val Ser Ser Gly
225 230 235 240

Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly
245 250 255

Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala
260 265 270

Ser Gly Phe Thr Phe Ser Asp Tyr Trp Met Tyr Trp Val Arg Gln Ala
275 280 285

Pro Gly Lys Gly Leu Glu Trp Val Ser Glu Ile Asn Thr Asn Gly Leu
290 295 300

Ile Thr Lys Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg
305 310 315 320

Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys Pro
325 330 335

Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg Ser Pro Ser Gly Phe Asn
340 345 350

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
355 360

<210> 91
<211> 391
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 91

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Glu Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Leu Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln Leu Val Glu
130 135 140

Ser Gly Gly Gly Leu Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys

145 150 155 160

Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro Ser Gly Tyr Thr Tyr Thr
165 170 175

Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
180 185 190

Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr Tyr Ala Asp Ser Val Lys
195 200 205

Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala Lys Asn Thr Val Asp Leu
210 215 220

Leu Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala
225 230 235 240

Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Gly Ser Tyr Asn Tyr
245 250 255

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser
260 265 270

Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val
275 280 285

Gln Pro Gly Asn Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
290 295 300

Phe Arg Ser Phe Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Glu
305 310 315 320

Pro Glu Trp Val Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr
325 330 335

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
340 345 350

Thr Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
355 360 365

Val Tyr Tyr Cys Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly
370 375 380

Thr Gln Val Thr Val Ser Ser
385 390

amended sequence listing1

<210> 92
<211> 379
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 92

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val
130 135 140

Gln Pro Gly Asn Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
145 150 155 160

Phe Arg Ser Phe Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Glu
165 170 175

Pro Glu Trp Val Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr
180 185 190

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
195 200 205

Thr Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala

210 215 220

Val Tyr Tyr Cys Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly
225 230 235 240

Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser
245 250 255

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
260 265 270

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser Asn Tyr
275 280 285

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu
290 295 300

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
305 310 315 320

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
325 330 335

Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
340 345 350

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
355 360 365

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
370 375

<210> 93
<211> 384
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 93

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
145 150 155 160

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp
165 170 175

Tyr Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
180 185 190

Val Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser
195 200 205

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu
210 215 220

Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr
225 230 235 240

Cys Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val
245 250 255

Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln
260 265 270

Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn Ser Leu Arg
275 280 285

Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe Gly Met Ser

290 295 300

Trp Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val Ser Ser Ile
305 310 315 320

Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val Lys Gly Arg
325 330 335

Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr Leu Gln Met
340 345 350

Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Thr Ile Gly
355 360 365

Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr Val Ser Ser
370 375 380

<210> 94
<211> 400
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 94

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
130 135 140

Gly Gly Ser Ser Gly Gly Gly Gly Ser Glu Val Gln Val Val Glu Ser
145 150 155 160

Gly Gly Gly Leu Val Gln Ala Gly Gly Ser Leu Ser Leu Ser Cys Ser
165 170 175

Ala Ser Gly Arg Ser Leu Ser Asn Tyr Tyr Met Gly Trp Phe Arg Gln
180 185 190

Ala Pro Gly Lys Glu Arg Glu Leu Leu Gly Asn Ile Ser Trp Arg Gly
195 200 205

Tyr Asn Ile Tyr Tyr Lys Asp Ser Val Lys Gly Arg Phe Thr Ile Ser
210 215 220

Arg Asp Asp Ala Lys Asn Thr Ile Tyr Leu Gln Met Asn Arg Leu Lys
225 230 235 240

Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala Ser Ile Leu Pro Leu
245 250 255

Ser Asp Asp Pro Gly Trp Asn Thr Tyr Trp Gly Gln Gly Thr Gln Val
260 265 270

Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln
275 280 285

Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn Ser Leu Arg
290 295 300

Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe Gly Met Ser
305 310 315 320

Trp Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val Ser Ser Ile
325 330 335

Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val Lys Gly Arg
340 345 350

Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr Leu Gln Met
355 360 365

Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Thr Ile Gly

370 375 380

Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr Val Ser Ser
385 390 395 400

<210> 95
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 95

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 96
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 96

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 97
<211> 129
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 97

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser

100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 98
<211> 129
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 98

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
115 120 125

Ser

<210> 99
<211> 123
<212> PRT
<213> Artificial

<220>

<223> nanobody construct

<400> 99

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 100
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 100

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr

65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 101
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 101

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 102
<211> 115
<212> PRT
<213> Artificial

<220>

amended sequence listing1

<223> nanobody construct

<400> 102

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 103
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 103

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr

65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 104
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 104

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 105
<211> 130
<212> PRT
<213> Artificial

<220>

<223> nanobody

<400> 105

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gln Ile Ile Phe Gly Ser His
20 25 30

Val Ala Ala Trp Phe Arg Gln Ala Pro Gly Arg Glu Arg Glu Phe Val
35 40 45

Ala Glu Ile Arg Pro Ser Gly Asp Phe Gly Pro Glu Gly Glu Phe Glu
50 55 60

His Val Thr Ala Ser Leu Lys Gly Arg Phe Thr Ile Ala Lys Asn Ser
65 70 75 80

Val Asp Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp
85 90 95

Thr Ala Val Tyr Tyr Cys Ala Ala Ala Pro Tyr Arg Gly Gly Arg Asp
100 105 110

Tyr Arg Trp Glu Tyr Glu Tyr Glu Tyr Trp Gly Gln Gly Thr Gln Val
115 120 125

Thr Val
130

<210> 106
<211> 122
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 106

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Asn Ala Gly Ser Thr Ser Asn Ala Tyr
20 25 30

Ala Thr Gly Trp Phe Arg Arg Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Gly Ile Gln Trp Ser Gly Gly Asp Ala Phe Tyr Arg Asn Ser Val

50 55 60

Lys Gly Arg Phe Arg Ile Thr Arg Asp Pro Asp Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Asp Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr Cys Ala
85 90 95

Gln Lys Leu Ser Pro Tyr Tyr Asn Asp Phe Asp Ser Ser Asn Tyr Glu
100 105 110

Tyr Trp Gly Gln Gly Thr Gln Val Thr Val
115 120

<210> 107
<211> 119
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 107

Glu Val Gln Leu Val Glu Ser Gly Gly Asp Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Gln Leu Phe Ser Thr Asn
20 25 30

Asp Val Gly Trp Tyr Arg Arg Ala Pro Gly Lys Gln Arg Glu Leu Val
35 40 45

Ala Thr Ile Thr Asp Asp Gly Thr Thr Asp Tyr Gly Asp Asp Val Lys
50 55 60

Gly Arg Phe Val Ile Ser Arg Glu Gly Glu Met Val Tyr Leu Glu Met
65 70 75 80

Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Asn Ile Asn
85 90 95

Arg Leu Arg Ser Thr Trp Gly Ile Arg Tyr Asp Val Trp Gly Gln Gly
100 105 110

Thr Gln Val Thr Val Ser Ser
115

<210> 108
<211> 115

<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 108

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Val Ser Gly Phe Thr Phe Ser Thr Thr
20 25 30

Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Phe Glu Glu Trp Val
35 40 45

Ser Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Gly Arg Arg Gly Tyr Gly Arg Asp Arg Ser Lys Gly Ile Gln Val Thr
100 105 110

Val Ala Ser
115

<210> 109
<211> 121
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 109

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Thr Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Phe Ser Ser Val
20 25 30

Ala Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gln Arg Glu Phe Leu
35 40 45

Ala Gly Val Gly Tyr Asp Gly Ser Ser Ile Arg Tyr Ala Glu Ser Val

50 55 60

Lys Gly Arg Phe Thr Ile Ala Arg Gly Asn Arg Glu Ser Thr Val Phe
65 70 75 80

Leu Gln Met Glu Asn Leu Lys Pro Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95

Thr Ala Glu Pro Ile Gly Ala Tyr Glu Gly Leu Trp Thr Tyr Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 110
<211> 124
<212> PRT
<213> Artificial

<220>
<223> nanobody

<220>
<221> misc_feature
<222> (1)..(4)
<223> unknown amino acid residues, probably EVQL, QVQL or a similar sequence

<400> 110

Xaa Xaa Xaa Xaa Val Glu Ser Gly Gly Gly Leu Met Gln Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Met Phe Ser Asp Ser
20 25 30

Ala Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Thr Ile Ser Trp Asn Gly Gly Ser Ser Ser Tyr Ala Asp Phe Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Gly Leu Thr Pro Gln Asp Thr Ala Ile Tyr Tyr Cys
85 90 95

Ala Gly Ser Tyr Ser Asn Gly Asn Pro His Arg Phe Ser Gln Tyr Gln
100 105 110

Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 111
<211> 120
<212> PRT
<213> Artificial

<220>
<223> nanobody

<220>
<221> MISC_FEATURE
<222> (77)..(77)
<223> Unknown amino acid residue

<400> 111

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Gly Thr Tyr
20 25 30

Ala Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Ala Ile Ser Trp Gly Gly Gly Ser Ile Val Tyr Ala Glu Ser Ala
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Xaa Thr Met Tyr
65 70 75 80

Leu Gln Met Asp Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ala Asn Asn Ile Ala Thr Leu Arg Gln Gly Ser Trp Gly Gln
100 105 110

Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 112
<211> 127
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 112

Glu Val Gln Leu Val Glu Ser Gly Gly Glu Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Arg Asn Phe Val Thr Tyr
20 25 30

Ala Met Ser Trp Phe Arg Arg Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Ser Ile Ser Trp Ser Gly Asp Thr Thr Tyr Tyr Ser Asn Ser Val
50 55 60

Lys Gly Arg Phe Thr Val Ser Arg Asp Asn Gly Lys Asn Thr Ala Tyr
65 70 75 80

Leu Arg Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Asp Tyr Tyr Cys
85 90 95

Ala Val Val Gln Val Ile Asp Pro Ser Trp Ser Gly Val Asn Leu Asp
100 105 110

Asp Tyr Asp Tyr Leu Gly Ser Gly Thr Gln Val Thr Val Ser Ser
115 120 125

<210> 113
<211> 124
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 113

Glu Val Gln Leu Val Glu Ser Gly Gly Arg Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Asn Ala Gly Ser Thr Ser Asn Ala Tyr
20 25 30

Ala Thr Gly Trp Phe Arg Arg Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Gly Ile Gln Trp Ser Gly Gly Asp Ala Phe Tyr Arg Asn Ser Val
50 55 60

Lys Gly Arg Phe Arg Ile Thr Arg Asp Pro Asp Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Asp Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr Cys Ala

85 90 95

Gln Lys Leu Ser Pro Tyr Tyr Asn Asp Phe Asp Ser Ser Asn Tyr Glu
100 105 110

Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 114
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 114

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Ala Thr Ile Ser Ser Ile Val
20 25 30

Met Leu Gly Trp Tyr Arg Gln Ala Pro Gly Lys Gln Arg Glu Trp Val
35 40 45

Ala Ser Ile Thr Ile Gly Ser Arg Thr Asn Tyr Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Phe Cys Asn
85 90 95

Ala Val Pro Pro Arg Asp Asp Tyr Trp Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 115
<211> 121
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 115

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Gln Thr Ser Ser Ser Tyr
20 25 30

Asp Met Gly Trp Phe Arg Gln Ala Pro Gly Glu Gly Arg Glu Phe Val
35 40 45

Ala Arg Ile Ser Gly Ser Asp Gly Ser Thr Tyr Tyr Ser Asp Arg Ala
50 55 60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Thr Lys Asn Met Val Tyr
65 70 75 80

Leu Gln Met Asp Arg Leu Lys Pro Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Arg Val Pro Arg Tyr Glu Asn Gln Trp Ser Ser Tyr Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 116
<211> 127
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 116

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ser Thr Phe Ser Thr Tyr
20 25 30

Asp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Asp Ser Gly Gly Gly Ser Pro Met Tyr Val Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Val Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys

85 90 95

Ala Lys Phe Ser Thr Gly Ala Asp Gly Gly Ser Trp Tyr Trp Ser Tyr
100 105 110

Gly Met Asp Ser Trp Gly Lys Gly Thr Gln Val Thr Val Ser Ser
115 120 125

<210> 117
<211> 121
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 117

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Glu Ala Ser Glu Arg Ser Ser Asn Arg Tyr
20 25 30

Asn Met Ala Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Leu
35 40 45

Ala Arg Val Asp Val Ser Gly Gly Asn Thr Leu Tyr Gly Asp Ser Val
50 55 60

Lys Asp Arg Phe Thr Val Ser Arg Ile Asn Gly Lys Asn Ala Met Tyr
65 70 75 80

Leu Gln Met Asn Asn Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr Cys
85 90 95

Ala Ala Gly Gly Trp Gly Thr Thr Gln Tyr Asp Tyr Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 118
<211> 117
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 118

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Cys Val Ser Ser Gly Cys Thr Phe Ser
20 25 30

Ala Tyr Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Ala Glu Glu
35 40 45

Trp Val Ser Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp
50 55 60

Ser Val Asn Gly Arg Phe Lys Ile Ser Arg Asp Asn Ala Lys Lys Thr
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Gly Pro Glu Asp Thr Ala Met Tyr
85 90 95

Tyr Cys Gln Arg Arg Gly Tyr Ala Leu Asp Arg Gly Gln Gly Thr Gln
100 105 110

Val Thr Val Ser Ser
115

<210> 119
<211> 129
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 119

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Thr Leu Ser Cys Ala Ser Ser Gly Arg Gly Phe Tyr Lys Asn
20 25 30

Ala Met Gly Trp Phe Arg Gln Pro Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Ser Ile Lys Trp Asn Gly Asn Asn Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Arg Gly Arg Phe Thr Ile Ser Arg Gly Asn Ala Lys Asn Thr Glu Asn
65 70 75 80

Thr Val Ser Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Asp

85 90 95

Tyr Tyr Cys Ala Ala Asp Ser Ser His Tyr Ser Tyr Val Tyr Ser Lys
100 105 110

Ala Tyr Glu Tyr Asp Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 120
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 120

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Phe Ser Gly Phe Ala Phe Ser Ala Ser
20 25 30

Ser Met Ala Trp Val Arg Gln Ala Pro Gly Lys Tyr Glu Glu Trp Val
35 40 45

Ser Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val
50 55 60

Gln Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Gly Arg Arg Gly Tyr Gly Arg Asp Arg Ser Gln Gly Ile Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 121
<211> 127
<212> PRT
<213> Artificial

<220>

<223> nanobody

<220>
<221> MISC_FEATURE
<222> (119)..(119)
<223> Unknown amino acid residue

<400> 121

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Ser Tyr
20 25 30

Ala Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Ala Ile Ser Trp Ser Gly Thr Ile Thr Asn Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Gly Lys Asn Thr Val His
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr His Cys
85 90 95

Ala Val Val Gln Pro Tyr Ser Gly Gly Asp Tyr Tyr Thr Gly Val Glu
100 105 110

Glu Tyr Asp Tyr Trp Gly Xaa Gly Thr Gln Val Thr Val Ser Ser
115 120 125

<210> 122
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 122

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Val Ser Gly Phe Thr Phe Ser Ala Thr
20 25 30

Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Ala Glu Glu Trp Val
35 40 45

Ser Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asp Asp Leu Gln Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Gly Arg Arg Gly Tyr Gly Arg Asp Arg Ser Arg Gly Ile Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 123
<211> 121
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 123

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Gly Ala Phe Ser Asn Tyr
20 25 30

Asp Val Gly Trp Phe Arg Gln Ala Pro Gly Glu Gly Arg Glu Ile Val
35 40 45

Ala Arg Ile Ser Gly Ser Gly Asp Ser Thr Tyr Ser Ser Asn Arg Ala
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Arg Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Arg Ala Ala Arg Tyr Asn Gly Thr Trp Ser Ser Asn Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 124
<211> 117
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 124

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Glu Cys Val Ser Ser Gly Cys Thr Phe Ser
20 25 30

Ala Tyr Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Ala Glu Glu
35 40 45

Phe Val Ser Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asn
50 55 60

Ser Val Asn Gly Arg Phe Lys Ile Ser Arg Asp Asn Ala Lys Lys Thr
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Gly Pro Glu Asp Thr Ala Met Tyr
85 90 95

Tyr Cys Gln Arg Arg Gly Tyr Ala Leu Asp Arg Gly Gln Gly Thr Gln
100 105 110

Val Thr Val Ser Ser
115

<210> 125
<211> 121
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 125

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Gln Thr Ser Ser Thr Ala
20 25 30

Asp Met Gly Trp Phe Arg Gln Pro Pro Gly Lys Gly Arg Glu Phe Val
35 40 45

Ala Arg Ile Ser Gly Ile Asp Gly Thr Thr Tyr Tyr Asp Glu Pro Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Lys Ala Gln Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asp Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Arg Ser Pro Arg Tyr Ala Asp Gln Trp Ser Ala Tyr Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 126
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 126

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Val Ser Gly Phe Thr Phe Ser Thr Thr
20 25 30

Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Phe Glu Glu Trp Val
35 40 45

Ser Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Gly Arg Arg Gly Tyr Gly Arg Asp Arg Ser Lys Gly Ile Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 127
<211> 118
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 127

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Ala Ser Ala Ser Gly Val Lys Val Asn
20 25 30

Asp Met Gly Trp Tyr Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Val
35 40 45

Ala Thr Ile Thr Asp Asp Gly Arg Thr Asn Tyr Glu Asp Phe Ala Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Leu Pro Glu Asp Thr Ala Val Tyr Tyr Cys Asn
85 90 95

Ala Arg Thr Tyr Trp Ala His Leu Pro Thr Tyr Trp Gly Gln Gly Thr
100 105 110

Gln Val Thr Val Ser Ser
115

<210> 128
<211> 121
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 128

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Phe Gly Ser Val
20 25 30

Ala Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Ala Ile Gly Tyr Asp Gly Asn Ser Ile Arg Tyr Gly Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ile Lys Asn Thr Met Tyr
65 70 75 80

Leu Glu Met Glu Asn Leu Asn Ala Asp Asp Thr Ala Arg Tyr Leu Cys
85 90 95

Ala Ala Glu Pro Leu Ala Arg Tyr Glu Gly Leu Trp Thr Tyr Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 129
<211> 121
<212> PRT
<213> Artificial

<220>
<223> nanobody

<400> 129

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Ala
1 5 10 15

Ser Leu Arg Leu Ser Cys Thr Thr Ser Thr Arg Thr Asn Asp Arg Phe
20 25 30

Asn Met Ala Trp Phe His Gln Ala Pro Gly Lys Asp Arg Glu Phe Val
35 40 45

Ser Arg Ile Asp Val Ala Gly Tyr Asn Thr Ala Tyr Gly Asp Phe Val
50 55 60

Lys Gly Arg Phe Thr Val Ser Arg Asp Ser Ala Glu Asn Thr Val Val
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Tyr Cys
85 90 95

Ala Ala Gly Gly Trp Gly Ile Ser Gln Ser Asp Tyr Asp Leu Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 130
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 130

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
20 25 30

<210> 131
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 131

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Met Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Gly
20 25 30

<210> 132
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 132

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Asp Phe Ser
20 25 30

<210> 133
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 133

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser
20 25 30

<210> 134
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 134

Gln Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser
20 25 30

<210> 135
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 135

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser
20 25 30

<210> 136
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 136

Ala Val Gln Leu Val Asp Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser
20 25 30

562

<210> 137
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 137

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ala Ala Ser Gly Arg Ser Phe Thr
20 25 30

<210> 138
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 138

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser
20 25 30

<210> 139
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 139

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gln Ile Ile Phe Gly
20 25 30

<210> 140
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 140

S63

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Asn Ala Gly Ser Thr Ser Asn
20 25 30

<210> 141
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 141

Glu Val Gln Leu Val Glu Ser Gly Gly Asp Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Gln Leu Phe Ser
20 25 30

<210> 142
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 142

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Val Ser Gly Phe Thr Phe Ser
20 25 30

<210> 143
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 143

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Thr Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Phe Ser
20 25 30

S64

<210> 144
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<220>
<221> MISC_FEATURE
<222> (1)..(4)
<223> Unknown amino acid residues, probably EVQL, QVQL or a similar sequence

<400> 144

Xaa Xaa Xaa Xaa Val Glu Ser Gly Gly Gly Leu Met Gln Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Met Phe Ser
20 25 30

<210> 145
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 145

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Gly
20 25 30

<210> 146
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 146

Glu Val Gln Leu Val Glu Ser Gly Gly Glu Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Arg Asn Phe Val
20 25 30

<210> 147
<211> 30

S65

<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 147

Glu Val Gln Leu Val Glu Ser Gly Gly Arg Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Asn Ala Gly Ser Thr Ser Asn
20 25 30

<210> 148
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 148

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Ala Thr Ile Ser Ser
20 25 30

<210> 149
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 149

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Gln Thr Ser Ser
20 25 30

<210> 150
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 150

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly

S66

1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ser Thr Phe Ser
20 25 30

<210> 151
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 151

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Glu Ala Ser Glu Arg Ser Ser Asn
20 25 30

<210> 152
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 152

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Cys Val Ser Ser Gly Cys Thr
20 25 30

<210> 153
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 153

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Thr Leu Ser Cys Ala Ser Ser Gly Arg Gly Phe Tyr
20 25 30

<210> 154
<211> 30

<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 154

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Phe Ser Gly Phe Ala Phe Ser
20 25 30

<210> 155
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 155

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser
20 25 30

<210> 156
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 156

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Val Ser Gly Phe Thr Phe Ser
20 25 30

<210> 157
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 157

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly

1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Gly Ala Phe Ser
20 25 30

<210> 158
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 158

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Glu Cys Val Ser Ser Gly Cys Thr
20 25 30

<210> 159
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 159

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Gln Thr Ser Ser
20 25 30

<210> 160
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 160

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Val Ser Gly Phe Thr Phe Ser
20 25 30

<210> 161
<211> 30

<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 161

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Ala Ser Ala Ser Gly Val Lys
20 25 30

<210> 162
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 162

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Phe Gly
20 25 30

<210> 163
<211> 29
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 163

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Ala
1 5 10 15

Ser Leu Arg Leu Ser Cys Thr Thr Ser Thr Arg Thr Asn
20 25

<210> 164
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 164

Asp Tyr Trp Met Tyr

1 5

<210> 165
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 165

Val Ser Trp Met Tyr
1 5

<210> 166
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 166

Val Ser Trp Met Tyr
1 5

<210> 167
<211> 10
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 167

Glu Pro Ser Gly Tyr Thr Tyr Thr Ile Gly
1 5 10

<210> 168
<211> 10
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 168

Asp Tyr Ser Gly Tyr Thr Tyr Thr Val Gly
1 5 10

<210> 169
<211> 10
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 169

Asp Tyr Ser Gly Tyr Thr Tyr Thr Val Gly
1 5 10

<210> 170
<211> 8
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 170

Ala His Ser Val Tyr Thr Met Gly
1 5

<210> 171
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 171

Gly Tyr Tyr Met Gly
1 5

<210> 172
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 172

Asn Tyr Tyr Met Gly
1 5

<210> 173
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 173

Ser His Val Ala Ala

1 5

<210> 174
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 174

Ala Tyr Ala Thr Gly
1 5

<210> 175
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 175

Thr Asn Asp Val Gly
1 5

<210> 176
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 176

Thr Thr Ser Met Thr
1 5

<210> 177
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 177

Ser Val Ala Met Gly
1 5

<210> 178
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 178

Asp Ser Ala Met Gly
1 5

<210> 179
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 179

Thr Tyr Ala Met Gly
1 5

<210> 180
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 180

Thr Tyr Ala Met Ser
1 5

<210> 181
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 181

Ala Tyr Ala Thr Gly
1 5

<210> 182
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 182

Ile Val Met Leu Gly

1 5

<210> 183
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 183

Ser Tyr Asp Met Gly
1 5

<210> 184
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 184

Thr Tyr Asp Met Ser
1 5

<210> 185
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 185

Arg Tyr Asn Met Ala
1 5

<210> 186
<211> 7
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 186

Phe Ser Ala Tyr Ser Met Thr
1 5

<210> 187
<211> 5
<212> PRT
<213> Artificial

575

<220>
<223> CDR1 element

<400> 187

Lys Asn Ala Met Gly
1 5

<210> 188
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 188

Ala Ser Ser Met Ala
1 5

<210> 189
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 189

Ser Tyr Ala Met Gly
1 5

<210> 190
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 190

Ala Thr Ser Met Thr
1 5

<210> 191
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 191

Asn Tyr Asp Val Gly

1 5

<210> 192
<211> 7
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 192

Phe Ser Ala Tyr Ser Met Thr
1 5

<210> 193
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 193

Thr Ala Asp Met Gly
1 5

<210> 194
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 194

Thr Thr Ser Met Thr
1 5

<210> 195
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 195

Val Asn Asp Met Gly
1 5

<210> 196
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 196

Ser Val Ala Met Gly
1 5

<210> 197
<211> 6
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 197

Asp Arg Phe Asn Met Ala
1 5

<210> 198
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 198

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
1 5 10

<210> 199
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 199

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
1 5 10

<210> 200
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 200

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser

1 5 10

<210> 201
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 201

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 202
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 202

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 203
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 203

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 204
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 204

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 205
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 205

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Gln Leu Leu Ala
1 5 10

<210> 206
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 206

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu Gly
1 5 10

<210> 207
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

400> 207

Trp Phe Arg Gln Ala Pro Gly Arg Glu Arg Glu Phe Val Ala
1 5 10

<210> 208
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 208

Trp Phe Arg Arg Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 209
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 209

Trp Tyr Arg Arg Ala Pro Gly Lys Gln Arg Glu Leu Val Ala

1 5 10

<210> 210
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 210

Trp Val Arg Gln Ala Pro Gly Lys Phe Glu Glu Trp Val Ser
1 5 10

<210> 211
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 211

Trp Phe Arg Gln Ala Pro Gly Lys Gln Arg Glu Phe Leu Ala
1 5 10

<210> 212
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 212

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 213
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 213

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 214
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 214

Trp Phe Arg Arg Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 215
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 215

Trp Phe Arg Arg Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 216
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 216

Trp Tyr Arg Gln Ala Pro Gly Lys Gln Arg Glu Trp Val Ala
1 5 10

<210> 217
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 217

Trp Phe Arg Gln Ala Pro Gly Glu Gly Arg Glu Phe Val Ala
1 5 10

<210> 218
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 218

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser

1 5 10

<210> 219
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 219

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Leu Ala
1 5 10

<210> 220
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 220

Trp Val Arg Gln Ala Pro Gly Lys Ala Glu Glu Trp Val Ser
1 5 10

<210> 221
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 221

Trp Phe Arg Gln Pro Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 222
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 222

Trp Val Arg Gln Ala Pro Gly Lys Tyr Glu Glu Trp Val Ser
1 5 10

<210> 223
<211> 14
<212> PRT
<213> Artificial

S83

<220>
<223> FR2 element

<400> 223

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 224
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 224

Trp Val Arg Gln Ala Pro Gly Lys Ala Glu Glu Trp Val Ser
1 5 10

<210> 225
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 225

Trp Phe Arg Gln Ala Pro Gly Glu Gly Arg Glu Ile Val Ala
1 5 10

<210> 226
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 226

Trp Val Arg Gln Ala Pro Gly Lys Ala Glu Glu Phe Val Ser
1 5 10

<210> 227
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 227

Trp Phe Arg Gln Pro Pro Gly Lys Gly Arg Glu Phe Val Ala

1 5 10

<210> 228
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 228

Trp Val Arg Gln Ala Pro Gly Lys Phe Glu Glu Trp Val Ser
1 5 10

<210> 229
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 229

Trp Tyr Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Val Ala
1 5 10

<210> 230
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 230

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 231
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 231

Trp Phe His Gln Ala Pro Gly Lys Asp Arg Glu Phe Val Ser
1 5 10

<210> 232
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 232

Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val Lys
1 5 10 15

Gly

<210> 233
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 233

Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val Lys
1 5 10 15

Gly

<210> 234
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 234

Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Val Asp Ser Val Lys
1 5 10 15

Gly

<210> 235
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 235

Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 236
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 236

Arg Ile Tyr Trp Ser Ser Gly Asn Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 237
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 237

Arg Ile Tyr Trp Ser Ser Gly Asn Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 238
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 238

Arg Ile Tyr Trp Ser Ser Ala Asn Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 239
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 239

Ser Ile Ser Trp Arg Gly Asp Asn Thr Tyr Tyr Lys Glu Ser Val Lys
1 5 10 15

Gly

<210> 240
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 240

Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val Lys
1 5 10 15

Gly

<210> 241
<211> 23
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 241

Glu Ile Arg Pro Ser Gly Asp Phe Gly Pro Glu Gly Glu Phe Glu His
1 5 10 15

Val Thr Ala Ser Leu Lys Gly
20

<210> 242
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 242

Gly Ile Gln Trp Ser Gly Gly Asp Ala Phe Tyr Arg Asn Ser Val Lys
1 5 10 15

Gly

<210> 243
<211> 16
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 243

Thr Ile Thr Asp Asp Gly Thr Thr Asp Tyr Gly Asp Asp Val Lys Gly
1 5 10 15

<210> 244
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 244

Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 245
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 245

Gly Val Gly Tyr Asp Gly Ser Ser Ile Arg Tyr Ala Glu Ser Val Lys
1 5 10 15

Gly

<210> 246
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 246

Thr Ile Ser Trp Asn Gly Gly Ser Ser Ser Tyr Ala Asp Phe Val Lys
1 5 10 15

Gly

<210> 247
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 247

Ala Ile Ser Trp Gly Gly Gly Ser Ile Val Tyr Ala Glu Ser Ala Lys
1 5 10 15

Gly

<210> 248
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 248

Ser Ile Ser Trp Ser Gly Asp Thr Thr Tyr Tyr Ser Asn Ser Val Lys
1 5 10 15

Gly

<210> 249
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 249

Gly Ile Gln Trp Ser Gly Gly Asp Ala Phe Tyr Arg Asn Ser Val Lys
1 5 10 15

Gly

<210> 250
<211> 16
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 250

Ser Ile Thr Ile Gly Ser Arg Thr Asn Tyr Ala Asp Ser Val Lys Gly
1 5 10 15

<210> 251
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 251

Arg Ile Ser Gly Ser Asp Gly Ser Thr Tyr Tyr Ser Asp Arg Ala Lys
1 5 10 15

Asp

<210> 252
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 252

Gly Ile Asp Ser Gly Gly Gly Ser Pro Met Tyr Val Asp Ser Val Lys
1 5 10 15

Gly

<210> 253
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 253

Arg Val Asp Val Ser Gly Gly Asn Thr Leu Tyr Gly Asp Ser Val Lys
1 5 10 15

Asp

<210> 254
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 254

Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val Asn
1 5 10 15

Gly

<210> 255
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 255

Ser Ile Lys Trp Asn Gly Asn Asn Thr Tyr Tyr Ala Asp Ser Val Arg
1 5 10 15

Gly

<210> 256
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 256

Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val Gln
1 5 10 15

Gly

<210> 257
<211> 17
<212> PRT

<213> Artificial

<220>
<223> CDR2 element

<400> 257

Ala Ile Ser Trp Ser Gly Thr Ile Thr Asn Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 258
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 258

Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 259
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 259

Arg Ile Ser Gly Ser Gly Asp Ser Thr Tyr Ser Ser Asn Arg Ala Lys
1 5 10 15

Gly

<210> 260
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 260

Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asn Ser Val Asn
1 5 10 15

S93

Gly

<210> 261
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 261

Arg Ile Ser Gly Ile Asp Gly Thr Thr Tyr Tyr Asp Glu Pro Val Lys
1 5 10 15

Gly

<210> 262
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 262

Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 263
<211> 16
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 263

Thr Ile Thr Asp Asp Gly Arg Thr Asn Tyr Glu Asp Phe Ala Lys Gly
1 5 10 15

<210> 264
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 264

Ala Ile Gly Tyr Asp Gly Asn Ser Ile Arg Tyr Gly Asp Ser Val Lys
1 5 10 15

Gly

<210> 265
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 265

Arg Ile Asp Val Ala Gly Tyr Asn Thr Ala Tyr Gly Asp Phe Val Lys
1 5 10 15

Gly

<210> 266
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 266

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg
20 25 30

<210> 267
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 267

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg
20 25 30

<210> 268
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 268

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asp Ser Leu Ile Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg
20 25 30

<210> 269
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 269

Arg Phe Thr Ile Ser Arg Asp Ile Ala Lys Asn Thr Val Asp Leu Leu
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 270
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 270

Arg Phe Thr Ile Ser Arg Asp Ile Ala Lys Asn Thr Val Asp Leu Leu
1 5 10 15

Met Asn Asn Leu Glu Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 271
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 271

Arg Phe Thr Ile Ser Arg Asp Ile Ala Lys Asn Thr Val Asp Leu Leu
1 5 10 15

Met Asn Asn Leu Glu Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 272
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 272

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Asp Leu Leu
1 5 10 15

Met Asn Cys Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 273
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 273

Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 274
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 274

Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr Leu Gln
1 5 10 15

Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

597

<210> 275
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 275

Arg Phe Thr Ile Ala Lys Asn Ser Val Asp Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 276
<211> 31
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 276

Arg Phe Arg Ile Thr Arg Asp Pro Asp Asn Thr Val Tyr Leu Gln Met
1 5 10 15

Asn Asp Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr Cys Ala Gln
20 25 30

<210> 277
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 277

Arg Phe Val Ile Ser Arg Glu Gly Glu Met Val Tyr Leu Glu Met Asn
1 5 10 15

Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Asn Ile
20 25 30

<210> 278
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 278

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Gly Arg
20 25 30

<210> 279
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 279

Arg Phe Thr Ile Ala Arg Gly Asn Arg Glu Ser Thr Val Phe Leu Gln
1 5 10 15

Met Glu Asn Leu Lys Pro Glu Asp Thr Ala Val Tyr Phe Cys Thr Ala
20 25 30

<210> 280
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 280

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Gly Leu Thr Pro Gln Asp Thr Ala Ile Tyr Tyr Cys Ala Gly
20 25 30

<210> 281
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<220>
<221> MISC_FEATURE
<222> (11)..(11)
<223> unknown amino acid residue

<400> 281

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Xaa Thr Met Tyr Leu Gln
1 5 10 15

Met Asp Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 282
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 282

Arg Phe Thr Val Ser Arg Asp Asn Gly Lys Asn Thr Ala Tyr Leu Arg
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Asp Tyr Tyr Cys Ala Val
20 25 30

<210> 283
<211> 31
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 283

Arg Phe Arg Ile Thr Arg Asp Pro Asp Asn Thr Val Tyr Leu Gln Met
1 5 10 15

Asn Asp Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr Cys Ala Gln
20 25 30

<210> 284
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 284

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Phe Cys Asn Ala
20 25 30

<210> 285

<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 285

Arg Phe Thr Ile Ser Arg Asp Asn Thr Lys Asn Met Val Tyr Leu Gln
1 5 10 15

Met Asp Arg Leu Lys Pro Asp Asp Thr Ala Val Tyr Tyr Cys Arg Val
20 25 30

<210> 286
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 286

Arg Phe Thr Val Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys
20 25 30

<210> 287
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 287

Arg Phe Thr Val Ser Arg Ile Asn Gly Lys Asn Ala Met Tyr Leu Gln
1 5 10 15

Met Asn Asn Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr Cys Ala Ala
20 25 30

<210> 288
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 288

Arg Phe Lys Ile Ser Arg Asp Asn Ala Lys Lys Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Gly Pro Glu Asp Thr Ala Met Tyr Tyr Cys Gln Arg
20 25 30

<210> 289
<211> 35
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 289

Arg Phe Thr Ile Ser Arg Gly Asn Ala Lys Asn Thr Glu Asn Thr Val
1 5 10 15

Ser Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Asp Tyr Tyr
20 25 30

Cys Ala Ala
35

<210> 290
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 290

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys Gly Arg
20 25 30

<210> 291
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 291

Arg Phe Thr Ile Ser Arg Asp Asn Gly Lys Asn Thr Val His Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr His Cys Ala Val

20 25 30

<210> 292
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 292

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asp Asp Leu Gln Ser Glu Asp Thr Ala Met Tyr Tyr Cys Gly Arg
20 25 30

<210> 293
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 293

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Arg Glu Asp Thr Ala Val Tyr Tyr Cys Arg Ala
20 25 30

<210> 294
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 294

Arg Phe Lys Ile Ser Arg Asp Asn Ala Lys Lys Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Gly Pro Glu Asp Thr Ala Met Tyr Tyr Cys Gln Arg
20 25 30

<210> 295
<211> 32
<212> PRT
<213> Artificial

<220>

<223> FR3 element

<400> 295

Arg Phe Thr Ile Ser Arg Asp Lys Ala Gln Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asp Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Arg Ser
20 25 30

<210> 296
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 296

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Gly Arg
20 25 30

<210> 297
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 297

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Leu Pro Glu Asp Thr Ala Val Tyr Tyr Cys Asn Ala
20 25 30

<210> 298
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 298

Arg Phe Thr Ile Ser Arg Asp Asn Ile Lys Asn Thr Met Tyr Leu Glu
1 5 10 15

Met Glu Asn Leu Asn Ala Asp Asp Thr Ala Arg Tyr Leu Cys Ala Ala

20 25 30

<210> 299
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 299

Arg Phe Thr Val Ser Arg Asp Ser Ala Glu Asn Thr Val Val Leu Gln
1 5 10 15

Met Asn Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 300
<211> 6
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 300

Ser Pro Ser Gly Phe Asn
1 5

<210> 301
<211> 6
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 301

Ser Pro Ser Gly Ser Phe
1 5

<210> 302
<211> 6
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 302

Ser Pro Ser Gly Ser Phe
1 5

<210> 303
<211> 15
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 303

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Gly Ser Tyr Asn Tyr
1 5 10 15

<210> 304
<211> 15
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 304

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Glu Ser Tyr Asn Tyr
1 5 10 15

<210> 305
<211> 15
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 305

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Glu Ser Tyr Asn Tyr
1 5 10 15

<210> 306
<211> 15
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 306

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Glu Ala Tyr Asn Tyr
1 5 10 15

<210> 307
<211> 14
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 307

Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Asn
1 5 10

<210> 308
<211> 14
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 308

Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
1 5 10

<210> 309
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 309

Ala Pro Tyr Arg Gly Gly Arg Asp Tyr Arg Trp Glu Tyr Glu Tyr Glu
1 5 10 15

Tyr

<210> 310
<211> 16
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 310

Lys Leu Ser Pro Tyr Tyr Asn Asp Phe Asp Ser Ser Asn Tyr Glu Tyr
1 5 10 15

<210> 311
<211> 13
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 311

Asn Arg Leu Arg Ser Thr Trp Gly Ile Arg Tyr Asp Val
1 5 10

<210> 312
<211> 6
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 312

Arg Gly Tyr Gly Arg Asp
1 5

<210> 313
<211> 12
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 313

Glu Pro Ile Gly Ala Tyr Glu Gly Leu Trp Thr Tyr
1 5 10

<210> 314
<211> 15
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 314

Ser Tyr Ser Asn Gly Asn Pro His Arg Phe Ser Gln Tyr Gln Tyr
1 5 10 15

<210> 315
<211> 11
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 315

Ala Asn Asn Ile Ala Thr Leu Arg Gln Gly Ser
1 5 10

<210> 316
<211> 18
<212> PRT

<213> Artificial

<220>
<223> CDR3 element

<400> 316

Val Gln Val Ile Asp Pro Ser Trp Ser Gly Val Asn Leu Asp Asp Tyr
1 5 10 15

Asp Tyr

<210> 317
<211> 16
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 317

Lys Leu Ser Pro Tyr Tyr Asn Asp Phe Asp Ser Ser Asn Tyr Glu Tyr
1 5 10 15

<210> 318
<211> 7
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 318

Val Pro Pro Arg Asp Asp Tyr
1 5

<210> 319
<211> 12
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 319

Pro Arg Tyr Glu Asn Gln Trp Ser Ser Tyr Asp Tyr
1 5 10

<210> 320
<211> 18
<212> PRT
<213> Artificial

<220>

<223> CDR3 element

<400> 320

Phe Ser Thr Gly Ala Asp Gly Gly Ser Trp Tyr Trp Ser Tyr Gly Met
1 5 10 15

Asp Ser

<210> 321
<211> 12
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 321

Gly Gly Trp Gly Thr Thr Gln Tyr Asp Tyr Asp Tyr
1 5 10

<210> 322
<211> 6
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 322

Arg Gly Tyr Ala Leu Asp
1 5

<210> 323
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 323

Asp Ser Ser His Tyr Ser Tyr Val Tyr Ser Lys Ala Tyr Glu Tyr Asp
1 5 10 15

Tyr

<210> 324
<211> 6
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 324

Arg Gly Tyr Gly Arg Asp
1 5

<210> 325
<211> 18
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 325

Val Gln Pro Tyr Ser Gly Gly Asp Tyr Tyr Thr Gly Val Glu Glu Tyr
1 5 10 15

Asp Tyr

<210> 326
<211> 6
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 326

Arg Gly Tyr Gly Arg Asp
1 5

<210> 327
<211> 12
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 327

Ala Arg Tyr Asn Gly Thr Trp Ser Ser Asn Asp Tyr
1 5 10

<210> 328
<211> 6
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 328

Arg Gly Tyr Ala Leu Asp
1 5

<210> 329
<211> 12
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 329

Pro Arg Tyr Ala Asp Gln Trp Ser Ala Tyr Asp Tyr
1 5 10

<210> 330
<211> 6
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 330

Arg Gly Tyr Gly Arg Asp
1 5

<210> 331
<211> 10
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 331

Arg Thr Tyr Trp Ala His Leu Pro Thr Tyr
1 5 10

<210> 332
<211> 12
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 332

Glu Pro Leu Ala Arg Tyr Glu Gly Leu Trp Thr Tyr
1 5 10

<210> 333

<211> 12
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 333

Gly Gly Trp Gly Ile Ser Gln Ser Asp Tyr Asp Leu
1 5 10

<210> 334
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 334

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 335
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 335

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 336
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 336

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 337
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 337

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 338
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 338

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 339
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 339

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 340
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 340

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 341
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 341

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 342

<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 342

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 343
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 343

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 344
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 344

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 345
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 345

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 346
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 346

Arg Ser Lys Gly Ile Gln Val Thr Val Ala Ser
1 5 10

<210> 347
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 347

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 348
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 348

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 349
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 349

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 350
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 350

Leu Gly Ser Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 351

<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 351

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 352
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 352

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 353
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 353

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 354
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 354

Trp Gly Lys Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 355
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 355

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 356
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 356

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 357
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 357

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 358
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 358

Arg Ser Gln Gly Ile Gln Val Thr Val Ser Ser
1 5 10

<210> 359
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<220>
<221> MISC_FEATURE
<222> (3)..(3)
<223> Unknown amino acid residue

<400> 359

Trp Gly Xaa Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 360
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 360

Arg Ser Arg Gly Ile Gln Val Thr Val Ser Ser
1 5 10

<210> 361
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 361

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 362
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 362

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 363
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 363

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 364
<211> 11

<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 364

Arg Ser Lys Gly Ile Gln Val Thr Val Ser Ser
1 5 10

<210> 365
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 365

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 366
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 366

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 367
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 367

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 368
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 368

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Gly Gly Gly
1 5 10 15

Ser Leu Arg Leu Ala Cys Ala Ala Ser Glu Arg Ile Phe Asp
20 25 30

<210> 369
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 369

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Glu Gly Gly
1 5 10 15

Ser Leu Arg Leu Ala Cys Ala Ala Ser Glu Arg Ile Trp Asp
20 25 30

<210> 370
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 370

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg
20 25 30

<210> 371
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 371

Ala Val Gln Leu Val Asp Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Phe Gly
20 25 30

<210> 372
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 372

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Thr Cys Thr Ala Ser Gly Phe Thr Phe Arg
20 25 30

<210> 373
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 373

Gln Val Gln Leu Ala Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Thr Cys Thr Ala Ser Gly Phe Thr Phe Gly
20 25 30

<210> 374
<211> 30
<212> PRT
<213> Artificial

<220>
<223> FR1 element

<400> 374

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
20 25 30

<210> 375
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 375

Leu Asn Leu Met Gly
1 5

<210> 376
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 376

Ile Asn Leu Leu Gly
1 5

<210> 377
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 377

Ser Phe Gly Met Ser
1 5

<210> 378
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 378

Ser Phe Gly Met Ser
1 5

<210> 379
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 379

Ser Phe Gly Met Ser
1 5

<210> 380
<211> 5

<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 380

Ser Phe Gly Met Ser
1 5

<210> 381
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR1 element

<400> 381

Asn Tyr Trp Met Tyr
1 5

<210> 382
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 382

Trp Tyr Arg Gln Gly Pro Gly Asn Glu Arg Glu Leu Val Ala
1 5 10

<210> 383
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 383

Trp Tyr Arg Gln Gly Pro Gly Asn Glu Arg Glu Leu Val Ala
1 5 10

<210> 384
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 384

Trp Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val Ser
1 5 10

<210> 385
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 385

Trp Val Arg Gln Tyr Pro Gly Lys Glu Pro Glu Trp Val Ser
1 5 10

<210> 386
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 386

Trp Val Arg Gln Ala Pro Gly Lys Asp Gln Glu Trp Val Ser
1 5 10

<210> 387
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 387

Trp Val Arg Gln Ala Pro Gly Glu Gly Leu Glu Trp Val Ser
1 5 10

<210> 388
<211> 14
<212> PRT
<213> Artificial

<220>
<223> FR2 element

<400> 388

Trp Val Arg Val Ala Pro Gly Lys Gly Leu Glu Arg Ile Ser
1 5 10

<210> 389
<211> 17

<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 389

Thr Cys Ile Thr Val Gly Asp Ser Thr Asn Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 390
<211> 16
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 390

Thr Ile Thr Val Gly Asp Ser Thr Ser Tyr Ala Asp Ser Val Lys Gly
1 5 10 15

<210> 391
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 391

Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 392
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 392

Ser Ile Asn Gly Arg Gly Asp Asp Thr Arg Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 393
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 393

Ala Ile Ser Ala Asp Ser Ser Thr Lys Asn Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 394
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 394

Ala Ile Ser Ala Asp Ser Ser Asp Lys Arg Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 395
<211> 18
<212> PRT
<213> Artificial

<220>
<223> CDR2 element

<400> 395

Arg Asp Ile Ser Thr Gly Gly Gly Tyr Ser Tyr Tyr Ala Asp Ser Val
1 5 10 15

Lys Gly

<210> 396
<211> 32
<212> PRT
<213> Artificial

<220>

<223> FR3 element

<400> 396

Arg Phe Thr Ile Ser Met Asp Tyr Thr Lys Gln Thr Val Tyr Leu His
1 5 10 15

Met Asn Ser Leu Arg Pro Glu Asp Thr Gly Leu Tyr Tyr Cys Lys Ile
20 25 30

<210> 397
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 397

Arg Phe Thr Ile Ser Arg Asp Tyr Asp Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Arg Pro Glu Asp Thr Gly Leu Tyr Tyr Cys Lys Ile
20 25 30

<210> 398
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 398

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Thr Ile
20 25 30

<210> 399
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 399

Arg Phe Ser Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Glu Tyr Tyr Cys Thr Ile

20 25 30

<210> 400
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 400

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Lys Met Leu Tyr Leu Glu
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Val Ile
20 25 30

<210> 401
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 401

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Lys Met Leu Tyr Leu Glu
1 5 10 15

Met Asn Ser Leu Lys Ser Glu Asp Thr Ala Val Tyr Tyr Cys Val Ile
20 25 30

<210> 402
<211> 32
<212> PRT
<213> Artificial

<220>
<223> FR3 element

<400> 402

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Lys
20 25 30

<210> 403
<211> 8
<212> PRT
<213> Artificial

<220>

<223> CDR3 element

<400> 403

Arg Arg Thr Trp His Ser Glu Leu
1 5

<210> 404
<211> 8
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 404

Arg Arg Thr Trp His Ser Glu Leu
1 5

<210> 405
<211> 6
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 405

Gly Gly Ser Leu Ser Arg
1 5

<210> 406
<211> 7
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 406

Gly Arg Ser Val Ser Arg Ser
1 5

<210> 407
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 407

Gly Arg Gly Ser Pro
1 5

<210> 408
<211> 5
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 408

Gly Arg Gly Ser Pro
1 5

<210> 409
<211> 13
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 409

Asp Arg Glu Ala Gln Val Asp Thr Leu Asp Phe Asp Tyr
1 5 10

<210> 410
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 410

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 411
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 411

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 412
<211> 11
<212> PRT
<213> Artificial

<220>

<223> FR4 element

<400> 412

Ser Ser Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 413
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 413

Arg Thr Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 414
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 414

Ser Ser Pro Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 415
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 415

Ala Ser Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 416
<211> 11
<212> PRT
<213> Artificial

<220>
<223> FR4 element

<400> 416

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 417
<211> 363
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 417

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln Leu
115 120 125

Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn Ser Leu Arg Leu
130 135 140

Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe Gly Met Ser Trp
145 150 155 160

Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ser Ile Ser
165 170 175

Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val Lys Gly Arg Phe
180 185 190

Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr Leu Gln Met Asn
195 200 205

Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys Thr Ile Gly Gly
210 215 220

Ser Leu Ser Arg Ser Ser Gln Gly Thr Leu Val Thr Val Ser Ser Gly
225 230 235 240

Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly
245 250 255

Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala
260 265 270

Ser Gly Phe Thr Phe Ser Asp Tyr Trp Met Tyr Trp Val Arg Gln Ala
275 280 285

Pro Gly Lys Gly Leu Glu Trp Val Ser Glu Ile Asn Thr Asn Gly Leu
290 295 300

Ile Thr Lys Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg
305 310 315 320

Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Pro
325 330 335

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Pro Ser Gly Phe Asn
340 345 350

Arg Gly Gln Gly Thr Leu Val Thr Val Ser Ser
355 360

<210> 418
<211> 379
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 418

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val

50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val
130 135 140

Gln Pro Gly Asn Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
145 150 155 160

Phe Ser Ser Phe Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly
165 170 175

Leu Glu Trp Val Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr
180 185 190

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
195 200 205

Thr Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala
210 215 220

Val Tyr Tyr Cys Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly
225 230 235 240

Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser
245 250 255

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
260 265 270

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Leu Ser Asn Tyr
275 280 285

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
290 295 300

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
305 310 315 320

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
325 330 335

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
340 345 350

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
355 360 365

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
370 375

<210> 419
<211> 260
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 419

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly

130 135 140

Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
145 150 155 160

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp
165 170 175

Tyr Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
180 185 190

Val Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser
195 200 205

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu
210 215 220

Tyr Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr
225 230 235 240

Cys Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val
245 250 255

Thr Val Ser Cys
260

<210> 420
<211> 264
<212> PRT
<213> Artificial

<220>
<223> nanobody construct

<400> 420

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
145 150 155 160

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp
165 170 175

Tyr Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
180 185 190

Val Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser
195 200 205

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu
210 215 220

Tyr Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr
225 230 235 240

Cys Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val
245 250 255

Thr Val Ser Ser Gly Gly Gly Cys
260

<210> 421
<211> 20
<212> DNA
<213> Artificial

<220>
<223> oligonucleotide primer

<400> 421
ggataacaat ttcacacagg 20

<210> 422

<211> 55
<212> DNA
<213> Artificial

<220>
<223> oligonucleotide primer

<400> 422
tcagtaacct ggatccgcca ccgctgcctc caccgcctga ggagacggtg accag 55

<210> 423
<211> 39
<212> DNA
<213> Artificial

<220>
<223> oligonucleotide primer

<400> 423
aggttactga ggatccgagg tgcagctggt ggagtctgg 39

<210> 424
<211> 46
<212> DNA
<213> Artificial

<220>
<223> oligonucleotide primer

<400> 424
tcagtaacct tccggaaccg ccaccgcctg aggagacggt gacaag 46

<210> 425
<211> 48
<212> DNA
<213> Artificial

<220>
<223> oligonucleotide primer

<400> 425
aggttactga tccggaggcg gtagcgaggt gcagctggtg gagtctgg 48

<210> 426
<211> 19
<212> DNA
<213> Artificial

<220>
<223> oligonucleotide primer

<400> 426
cacgacgttg taaaacgac 19

<210> 427
<211> 43
<212> DNA
<213> Artificial

<220>
<223> oligonucleotide primer

<400> 427
atggtggtgt gcggccgcct attatgagga gacggtgacc agg 43

<210> 428
<211> 45
<212> DNA
<213> Artificial

<220>
<223> oligonucleotide primer

<400> 428
aggggtatct ctcgagaaaa gagaggtgca gctggtggag tctgg 45

<210> 429
<211> 56
<212> DNA
<213> Artificial

<220>
<223> oligonucleotide primer

<400> 429
gctaaagaag aaggggtatc tctcgagaaa agagaggtgc agctggtgga gtctgg 56

<210> 430
<211> 88
<212> DNA
<213> Artificial

<220>
<223> oligonucleotide primer

<400> 430
tcagtaacct ggatcccccg ccaccgctgc ctccaccgcc gctacccccg ccaccgctgc 60

ctccaccgcc tgaggagacg gtgacaag 88

<210> 431
<211> 69
<212> DNA
<213> Artificial

<220>
<223> oligonucleotide primer

<400> 431
aggttactga ggatccggcg gtggaggcag cggtggcggg ggtagcgagg tgcagctggt 60

ggagtctgg 69

<210> 432
<211> 41
<212> DNA

<213> Artificial

<220>
<223> oligonucleotide primer

<400> 432
atggtggtgt gaattcttat tagcaggaga cggtgacaag g 41

<210> 433
<211> 53
<212> DNA
<213> Artificial

<220>
<223> oligonucleotide primer

<400> 433
atggtggtgt gaattcttat tagcaacctc cacctgagga gacggtgaca agg 53

<210> 434
<211> 21
<212> DNA
<213> Artificial

<220>
<223> oligonucleotide primer

<400> 434
gactggttcc aattgacaag c 21

<210> 435
<211> 21
<212> DNA
<213> Artificial

<220>
<223> oligonucleotide primer

<400> 435
gcaaatggca ttctgacatc c 21

<210> 436
<211> 13
<212> PRT
<213> Artificial

<220>
<223> CDR3 element

<400> 436

Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
1 5 10

<210> 437
<211> 4
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 437

Lys Glu Arg Glu
1

<210> 438
<211> 4
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 438

Lys Gln Arg Glu
1

<210> 439
<211> 4
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 439

Gly Leu Glu Trp
1

<210> 440
<211> 5
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 440

Lys Glu Arg Glu Leu
1 5

<210> 441
<211> 5
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 441

Lys Glu Arg Glu Phe

1 5

<210> 442
<211> 5
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 442

Lys Gln Arg Glu Leu
1 5

<210> 443
<211> 5
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 443

Lys Gln Arg Glu Phe
1 5

<210> 444
<211> 5
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 444

Lys Glu Arg Glu Gly
1 5

<210> 445
<211> 4
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 445

Thr Glu Arg Glu
1

<210> 446
<211> 5
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 446

Thr Glu Arg Glu Leu
1 5

<210> 447
<211> 4
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 447

Lys Glu Cys Glu
1

<210> 448
<211> 5
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 448

Lys Glu Cys Glu Leu
1 5

<210> 449
<211> 5
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 449

Lys Glu Cys Glu Arg
1 5

<210> 450
<211> 4
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 450

Arg Glu Arg Glu

1

<210> 451
<211> 5
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 451

Arg Glu Arg Glu Gly
1 5

<210> 452
<211> 4
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 452

Gln Glu Arg Glu
1

<210> 453
<211> 5
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 453

Gln Glu Arg Glu Gly
1 5

<210> 454
<211> 4
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 454

Lys Gly Arg Glu
1

<210> 455
<211> 5
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 455

Lys Gly Arg Glu Gly
1 5

<210> 456
<211> 4
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 456

Lys Asp Arg Glu
1

<210> 457
<211> 5
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 457

Lys Asp Arg Glu Val
1 5

<210> 458
<211> 5
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 458

Asp Glu Cys Lys Leu
1 5

<210> 459
<211> 5
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 459

Asn Val Cys Glu Leu

1 5

<210> 460
<211> 4
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 460

Gly Val Glu Trp
1

<210> 461
<211> 4
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 461

Glu Pro Glu Trp
1

<210> 462
<211> 4
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> .462

Gly Leu Glu Arg
1

<210> 463
<211> 4
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 463

Asp Gln Glu Trp
1

<210> 464
<211> 4
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 464

Asp Leu Glu Trp
1

<210> 465
<211> 4
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 465

Gly Ile Glu Trp
1

<210> 466
<211> 4
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 466

Glu Leu Glu Trp
1

<210> 467
<211> 4
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 467

Gly Pro Glu Trp
1

<210> 468
<211> 4
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 468

Glu Trp Leu Pro

1

<210> 469
<211> 4
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 469

Gly Pro Glu Arg
1

<210> 470
<211> 4
<212> PRT
<213> Artificial

<220>
<223> nanobody element

<400> 470

Gly Leu Glu Arg
1

<210> 471
<211> 4
<212> PRT
<213> Artificial

<220>
<223> spacer

<400> 471

Gly Gly Gly Cys
1

<210> 472
<211> 109
<212> PRT
<213> Artificial

<220>
<223> immunoglobulin construct

<400> 472

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Ser Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105

<210> 473
<211> 109
<212> PRT
<213> Artificial

<220>
<223> immunoglobulin construct

<400> 473

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Trp Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Val Trp Val
35 40 45

Ser Arg Ile Asn Ser Asp Gly Ser Ser Thr Ser Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105

<210> 474
<211> 109
<212> PRT
<213> Artificial

<220>
<223> immunoglobulin construct

<400> 474

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105

<210> 475
<211> 111
<212> PRT
<213> Artificial

<220>
<223> immunoglobulin construct

<400> 475

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp His
20 25 30

Tyr Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Arg Thr Arg Asn Lys Ala Asn Ser Tyr Thr Thr Glu Tyr Ala Ala
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Ala Arg Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105 110

<210> 476
<211> 17
<212> PRT
<213> Artificial

<220>
<223> linker

<400> 476

Ala Ala Ala Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Gly Ala
1 5 10 15

Ala

NANOBODIES™ (NANOCUERPOS) MEJORADOS CONTRA EL FACTOR ALFA DE NECROSIS DEL TUMOR

CAMPO DE LA INVENCIÓN

La presente invención se refiere a Nanobodies™ (Nanocuerpos) mejorados contra el factor-alfa de necrosis del tumor (TNF-alfa), así como también a polipéptidos que comprenden o consisten esencialmente de uno o más de tales Nanocuerpos. [Nota: *Nanobody™, Nanobodies™ y Nanoclone™, son marcas registradas de Ablynx N.V.*]

ANTECEDENTES DE LA INVENCIÓN

La invención también se refiere a ácidos nucleicos que codifican tales Nanocuerpos y polipéptidos; a métodos para preparar tales Nanocuerpos y polipéptidos; a células hospederas que expresan o son capaz de expresar tales Nanocuerpos o polipéptidos; a composiciones que comprenden tales Nanocuerpos, polipéptidos, ácidos nucleicos o células hospederas; y a usos de tales Nanocuerpos, tales polipéptidos, tales ácidos nucleicos, tales células hospederas o tales composiciones, en particular, para propósitos profilácticos, terapéuticos o de diagnóstico, tales como los propósitos profilácticos, terapéuticos o de diagnóstico mencionados posteriormente.

Otros aspectos, modalidades, ventajas y

aplicaciones de la invención, llegarán a ser más claros a partir de la descripción adicional aquí posteriormente.

El documento WO 04/041862 por el solicitante, se refiere a Nanocuerpos contra TNF-alfa y a la preparación y uso de los mismos, en particular, para la prevención y/o tratamiento de enfermedades y trastornos asociados con y/o mediados por TNF-alfa, tales como inflamación, artritis reumatoide, enfermedad de Crohn, colitis ulcerativa, síndrome del intestino inflamatorio, esclerosis múltiple, enfermedad de Addison, hepatitis autoinmune, parotitis autoinmune, diabetes tipo I, epididimitis, glomerulonefritis, enfermedad de Graves, síndrome Guillain-Barre, enfermedad de Hashimoto, anemia hemolítica, lupus eritematoso sistémico, infertilidad masculina, esclerosis múltiple, miastenia gravis, pénfigo, psoriasis, fiebre reumática, artritis reumatoide, sarcoidosis, escleroderma, síndrome de Sjogren, espondiloartropatías, tiroiditis y vasculitis.

Los Nanocuerpos anti-TNF de conformidad con el documento WO 04/041862, pueden ser humanizados y pueden ser monovalentes o multivalentes, el último de los cuales conduce a afinidad incrementada para TNF. Los Nanobodies™ (nanocuerpos) anti-TNF de conformidad con el documento WO 04/041862, pueden también ser multiespecíficos, y pueden estar en particular, en la forma de constructos

multiespecíficos que comprenden dos o más Nanocuerpos contra TNF y un Nanocuerpo adicional dirigido contra una proteína del suero, tal como albúmina de suero humana, lo cual conduce a una vida media in vivo incrementada.

El documento WO 04/041862, también se refiere a métodos para la preparación de los Nanocuerpos anti-TNF, a ácidos nucleicos o constructos que codifican los Nanocuerpos anti-TNF, así como también a composiciones farmacéuticas que comprenden los Nanocuerpos anti-TNF, los cuales pueden ser adecuados para administración intravenosa, subcutánea, oral, sublingual, tópica, nasal, vaginal o rectal, o para administración por inhalación. Los Nanocuerpos anti-TNF de conformidad con el documento WO 04/041862, pueden ser usados para propósitos de diagnóstico, opcionalmente en la forma de un kit de partes.

El documento EP 486 526, describe ligandos de enlace TNF-alfa contra un epítope específico de TNF. Entre los ligandos de enlace, se mencionan los anticuerpos de dominio único ("dAbs").

Reiter et al., *J. Mol. Biol.* (1999), 290, 685-698, describe anticuerpos de dominio único contra TNF-alfa, obtenidos a partir de una biblioteca de exhibición de fago aleatorizada, que se generó partiendo de un andamiaje de dominio VH a partir de un hibridoma de ratón.

El documento WO 00/29004, describe anticuerpos de

dominio único de murina ("microcuerpos"), contra TNF-alfa.

El documento WO 04/003019 *entre otras cosas*, describe ligandos que comprenden un primer dominio de enlace contra TNF-alfa y un segundo dominio de enlace contra una proteína del suero, tal como albúmina de suero.

SUMARIO DE LA INVENCIÓN

Es un objeto general de la presente invención, proporcionar Nanocuerpos contra TNF-alfa, en particular, contra TNF-alfa humano.

En particular, es un objeto de la presente invención, proporcionar Nanocuerpos contra TNF-alfa, en particular, contra TNF-alfa humano, y proporcionar proteínas o polipéptidos que comprenden los mismos, que son adecuados para uso terapéutico y/o de diagnóstico, y en particular, para la prevención, tratamiento y/o diagnóstico de una o más enfermedades y trastornos asociados con y/o mediados por TNF-alfa, tal como aquellos mencionados anteriormente, y/o que pueden ser usados en la preparación de una composición farmacéutica para la prevención y/o tratamiento de una o más enfermedades asociadas con y/o mediadas por TNF-alfa, tales como aquellas mencionadas anteriormente.

Más en particular, es un objeto de la invención, proporcionar Nanocuerpos contra TNF-alfa, y proporcionar

proteínas y polipéptidos que comprenden los mismos, que son ya sea una alternativa a los Nanocuerpos y polipéptidos contra TNF-alfa descrito en el documento WO 04/041862 y/o que tienen una o más propiedades o características mejoradas, comparadas con los Nanocuerpos y polipéptidos contra TNF-alfa descrito en el documento WO 04/041862.

Más en particular, es un objeto de la invención, proporcionar Nanocuerpos contra TNF-alfa, y proporcionar proteínas o polipéptidos que comprenden el mismo, que son mejorados comparados con los Nanocuerpos y polipéptidos contra TNF-alfa descrito en el documento WO 04/041862, con respecto a una o más de las siguientes propiedades o características:

- afinidad incrementada para TNF-alfa, ya sea en un formato monovalente, en un formato multivalente (por ejemplo, en un formato bivalente) y/o en un formato multiespecífico (por ejemplo, uno de los formatos multiespecíficos descritos en el documento WO 04/041862 o aquí posteriormente);
- mejor adecuabilidad para formatear en un formato multivalente (por ejemplo, en un formato bivalente);
- mejor adecuabilidad para formatear en un formato multiespecífico (por ejemplo, uno de los formatos multiespecíficos descritos en el documento WO 04/041862 o aquí posteriormente);

- adecuabilidad o susceptibilidad mejorada para sustituciones "humanizantes" (como se define en este documento); y/o
- menos inmunogenicidad, ya sea en un formato monovalente, en un formato multivalente (por ejemplo, en un formato bivalente) y/o en un formato multiespecífico (por ejemplo, uno de los formatos multiespecíficos descritos en el documento WO 04/041862 o aquí posteriormente), en un formato monovalente;
- estabilidad incrementada, ya sea en un formato monovalente, en un formato multivalente (por ejemplo, en un formato bivalente) y/o en un formato multiespecífico (por ejemplo, uno de los formatos multiespecíficos descritos en el documento WO 04/041862 o aquí posteriormente), en un formato monovalente;
- especificidad incrementada hacia TNF-alfa, ya sea en un formato monovalente, en un formato multivalente (por ejemplo, en un formato bivalente) y/o en un formato multiespecífico (por ejemplo, uno de los formatos multiespecíficos descritos en el documento WO 04/041862 o aquí posteriormente), en un formato monovalente;
- actividad cruzada reducida o en donde se desee,

incrementada con TNF-alfa a partir de especies diferentes;

y

- una o más propiedades mejoradas deseables para uso farmacéutico (que incluye, uso profiláctico y/o uso terapéutico) y/o para uso de diagnóstico (que incluye pero no se limita al uso para propósitos de formación de imagen), ya sea en un formato monovalente, en un formato multivalente (por ejemplo, en un formato bivalente) y/o en un formato multiespecífico (por ejemplo, uno de los formatos multiespecíficos descritos en el documento WO 04/041862 o aquí posteriormente).

Estos objetos se logran por Nanocuerpos, proteínas y polipéptidos descritos en este documento. Estos Nanocuerpos también son referidos en este documento como *"Nanocuerpos de la invención"*; y estas proteínas y polipéptidos, también son colectivamente referidos aquí como *"polipéptidos de la invención"*.

Puesto que los Nanocuerpos y polipéptidos descritos en este documento son principalmente propuestos para uso terapéutico y/o de diagnóstico, se dirigen contra (como se define aquí), TNF-alfa humano. Sin embargo, no se excluye (pero también no se requiere), que los Nanocuerpos y polipéptidos descritos en este documento, muestren

reactividad cruzada con TNF-alfa a partir de una o más de otras especies de animales de sangre caliente, por ejemplo, con TNF-alfa a partir de una o más de otras especies de primates y/o con TNF-alfa a partir de una o más especies de animales que son a menudo, usados en modelos de animal para enfermedades (por ejemplo, ratón, rata, conejo, cerdo o perro), y en particular, en modelos animales para enfermedades y trastornos asociados con TNF-alfa (tal como las especies y modelos animales mencionados en este documento). En este sentido, será claro para las personas expertas, que tal reactividad cruzada, cuando se presenta, puede tener ventajas a partir de un punto de vista de desarrollo de fármaco, puesto que permite a los Nanocuerpos y polipéptidos, contra el TNF-alfa humano, ser probados en tales modelos de enfermedad.

La presente invención está en su sentido más amplio también no limitada particularmente a, o definida por un determinante antigénico específico, epítope, parte, dominio, subunidad o confirmación (en donde sea aplicable) de TNF-alfa contra la cual los Nanocuerpos y polipéptidos de la invención se dirigen.

Sin embargo, en una modalidad preferida, los Nanocuerpos, proteínas o polipéptidos descritos en este documento, se dirigen contra y/o pueden enlazarse a un epítope de TNF-alfa que cae en, y/o forma parte del(los)

sitio(s) de enlace del receptor TNF (por ejemplo, los sitios de enlace para el TNF-RI, THF-RII, también conocidos como p55 ó p75). Como es bien sabido en la técnica, un trímero de TNF comprende tres sitios de enlace al receptor, los cuales son esencialmente equivalentes y los cuales son formados por/en la interfaz de dos monómeros de TNF con el trímero de TNF. Por ejemplo, los Nanocuerpos, proteínas o polipéptidos descritos en este documento, son preferiblemente dirigidos contra, y/o pueden enlazarse a un epítope de TNF-alfa que comprende los siguientes residuos de aminoácido de TNF-alfa: Gln en la posición 88, Lys en la posición 90, y/o Glu en la posición 146).

En particular, los Nanocuerpos, proteínas o polipéptidos descritos en este documento, son dirigidos contra y/o pueden enlazarse a un epítope del trímero de TNF-alfa, el cual confía en, y/o forma parte del(los) sitio(s) de enlace al receptor de TNF. Por ejemplo, los Nanocuerpos, proteínas o polipéptidos descritos aquí, pueden ser dirigidos contra y/o pueden enlazarse a un epítope del trímero de TNF-alfa que comprende los siguientes residuos de aminoácido: Gln en la posición 88 y Lys en la posición 90 en un primer monómero de TNF (referido en este documento como "monómero A"), y Glu en la posición 146 en un segundo monómero de TNF (referido aquí como "monómero B") (en el cual, el monómero A y monómero B

juntos, en el trímero de TNF, forman el(los) sitio(s) de enlace al receptor de TNF).

Más particularmente, los Nanocuerpos, proteínas o polipéptidos descritos en este documento, pueden ser dirigidos contra y/o pueden enlazarse a un epítope del trímero de TNF-alfa, que comprende los aminoácidos mencionados anteriormente (Gln en la posición 88 en el monómero A; Lys en la posición 90 en el monómero A y Glu en la posición 146 en el monómero B), y además, al menos uno, preferiblemente dos o más, más preferiblemente 5 ó más, y preferiblemente todo o esencialmente todos, los siguientes residuos de aminoácido del monómero de TNF-alfa A: Gly en la posición 24, Gln en la posición 25, Thr en la posición 72, His en la posición 73, Val en la posición 74, Leu en la posición 75, Thr en la posición 77, Thr en la posición 79, Ile en la posición 83, Thr en la posición 89, Val en la posición 91, Asn en la posición 92, Ile en la posición 97, Arg en la posición 131, Glu en la posición 135, Ile en la posición 136, Asn en la posición 137, Arg en la posición 138, Pro en la posición 139, Asp en la posición 140 y los siguientes residuos en el monómero B: Pro en la posición 20, Arg en la posición 32, Lys en la posición 65, Lys en la posición 112, Tyr en la posición 115, Ala en la posición 145, Ser en la posición 147.

Alternativamente, los Nanocuerpos, proteínas o

polipéptidos descritos en este documento, pueden ser dirigidos contra y/o pueden enlazarse a un epítope de TNF-alfa, que comprende los aminoácidos mencionados anteriormente (Gln en la posición 88 en el monómero A; Lys en la posición 90 en el monómero A y Glu en la posición 146 en el monómero B), y además, al menos uno, preferiblemente dos o más, más preferiblemente 5 ó más, y preferiblemente todo o esencialmente todos, los siguientes residuos de aminoácido del monómero de TNF-alfa A: Leu en la posición 75, Thr en la posición 77, Thr en la posición 79, Ile en la posición 80, Ser en la posición 81, Tyr en la posición 87, Thr en la posición 89, Val en la posición 91, Asn en la posición 92, Ser en la posición 95, Ile en la posición 97, Glu en la posición 135, Ile en la posición 136, Asn en la posición 137, y los siguientes residuos en el monómero B: Ala en la posición 33, Ala en la posición 145, Ser en la posición 147.

Tales epítopes pueden ser delineados de análisis estructural del nanocuerpo cristalizado en el complejo con la molécula de TNF, o de otros procedimientos tales como el mapeo de epítope vía análisis de pepscan.

Por comparación, de los datos cristalográficos (no mostrados), se puede observar que el Nanocuerpo 3E del documento WO 04/041862 enlazado a un epítope diferente (es decir, un epítope que comprende Tyr en la posición 141, Asp

en la posición 140, Gln en la posición 67, Gly en la posición 24 y Glu en la posición 23), que el epítope preferido de la invención.

De este modo, en otro aspecto, la presente invención se refiere a un dominio variable de inmunoglobulina (o un fragmento adecuado del mismo), que puede enlazarse a un epítope de TNF-alfa que cae en, y/o forma parte del sitio de enlace al receptor de TNF, y preferiblemente, a un epítope que comprende al menos, uno, preferiblemente dos o más, y preferiblemente, todos de los siguientes residuos de aminoácido de TNF-alfa: Gln en la posición 88; Lys en la posición 90 y Glu en la posición 146. Tal dominio variable de inmunoglobulina, es preferiblemente un dominio variable de cadena pesada o un dominio variable de cadena ligera, y en particular, un dominio variable de cadena pesada, el cual puede ser cualquiera de un dominio variable de cadena pesada de mamífero, que incluye pero no se limita a, dominios variables de cadena pesada de humano, dominios variables de cadena pesada de ratón y dominios variables de cadena pesada de Camélidos (tales como los dominios variables de cadena pesada a partir de inmunoglobulinas de 4 cadenas de Camélidos o los dominios variables de cadena pesada (dominios de VHH), de los así llamados anticuerpos de cadena pesada). El dominio variable de inmunoglobulina, es

664

preferiblemente, un anticuerpo de dominio o un anticuerpo de dominio único o adecuado para uso como un anticuerpo de dominio (único). Más preferiblemente, el dominio variable de inmunoglobulina es un Nanocuerpo (como se define aquí), y más preferido, pero sin ejemplos limitantes de Nanocuerpos que son adecuados para uso en este aspecto de la invención son, PMP1C2 (TNF1, SEC ID No: 52) y PMP5F10 (TNF3, SEC ID NO: 60), así como también las variantes humanizadas y otras de los mismos (como se describe aquí adicionalmente).

El dominio variable de inmunoglobulina mencionado anteriormente, también puede ser humanizado (como por ejemplo, y sin limitación), descrito aquí con respecto a Nanocuerpos. La invención también se refiere a proteínas y polipéptidos que comprenden o consisten esencialmente de tales dominios variables de inmunoglobulina, los cuales pueden por ejemplo, ser como se definen en este documento. Alternativamente, tales dominios de variables pueden formar parte de constructos de ScFv, constructos específicos duales, estructuras de anticuerpo o anticuerpo quimérico y otros constructos de inmunoglobulina, como por ejemplo, se revisa por Hoogenboom (Nature Biotechnology (1997), 15:125-126). Preferiblemente, sin embargo, los dominios variables de inmunoglobulina dirigidos contra el epítope anterior, son Nanocuerpos, en tal caso, las proteínas y polipéptidos

665

que comprenden tales Nanocuerpos, pueden ser como se describen adicionalmente en este documento.

De este modo, algunos aspectos preferidos de la invención se refieren a:

I) Un Nanocuerpo el cual se dirige contra el mismo epítope en el trímero de TNF-alfa como Nanocuerpo TNF1 (SEC ID NO: 52).

II) Un Nanocuerpo el cual se dirige contra el mismo epítope en el trímero de TNF-alfa como Nanocuerpo TNF3 (SEC ID NO: 60).

III) Un Nanocuerpo el cual se dirige contra un epítope del trímero de TNF-alfa que al menos comprenden los siguientes residuos de aminoácidos: Gln en la posición 88 en el monómero A; Lys en la posición 90 en el monómero A y Glu en la posición 146 en el monómero B.

IV) Un Nanocuerpo el cual se dirige contra un epítope del trímero de TNF-alfa que comprende los siguientes residuos de aminoácidos: Gln en la posición 88 en el monómero A; Lys en la posición 90 en el monómero A y Glu en la posición 146 en el monómero B; y que además comprende al menos, uno, preferiblemente dos o más, más preferiblemente 5 o más, y preferiblemente todos o esencialmente todos, de los siguientes residuos de aminoácidos del monómero A de TNF-alfa: Gly en la posición 24, Gln en la posición 25, Thr en la posición

666

72, His en la posición 73, Val en la posición 74, Leu en la posición 75, Thr en la posición 77, Thr en la posición 79, He en la posición 83, Thr en la posición 89, Val en la posición 91. Asn en la posición 92, He en la posición 97, Arg en la posición 131, Glu en la posición 135, He en la posición 136, Asn en la posición 137, Arg en la posición 138, Pro en la posición 139, Asp en la posición 140 y los siguientes residuos en el monómero B: Pro en la posición 20, Arg en la posición 32, Lys en la posición 65, Lys en la posición 112, Tyr en la posición 1 15, Ala en la posición 145, Ser en la posición 147.

V) Un Nanocuerpo el cual se dirige contra un epítope del trímero de TNF-alfa que comprende los siguientes residuos de aminoácidos: Gln en la posición 88 en el monómero A; Lys en la posición 90 en el monómero A y Glu en la posición 146 en el monómero B; y que además comprende al menos, uno, preferiblemente dos o más, más preferiblemente 5 ó más, y preferiblemente todos o esencialmente todos, de los siguientes residuos de aminoácidos del monómero A de TNF-alfa: Leu en la posición 75, Thr en la posición 77, Thr en la posición 79, He en la posición 80, Ser en la posición 81, Tyr en la posición 87, Thr en la posición 89, Val en la posición 91, Asn en la posición 92, Ser en la posición

667

95, He en la posición 97, Glu en la posición 135, He en la posición 136, Asn en la posición 137 y los siguientes residuos en el monómero B: Ala en la posición 33, Ala en la posición 145, Ser en la posición 147.

- Un Nanocuerpo de conformidad con cualquiera de I) a V) anteriores, los cuales tienen una relación K_{off} para TNF mejor de 2.10^{-3} (1/s), preferiblemente mejor que 1.10^{-3}.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, los cuales tienen un valor EC50 en el ensayo a base de células, usando las células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que el valor EC50 del Nanocuerpo VHH 3E (SEC ID NO:4) del documento WO 04/041862 en el mismo ensayo; o una variante humanizada de tal Nanocuerpo.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, los cuales tienen un valor EC50 en el ensayo a base de células, usando las células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que 12nM; o una variante humanizada de tal Nanocuerpo.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, los cuales tienen un valor EC50 en el

668

ensayo a base de células, usando las células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que 5nM; o una variante humanizada de tal Nanocuerpo.

- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, los cuales tienen un valor EC50 en el ensayo a base de células, usando las células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que 3nM; o una variante humanizada de tal Nanocuerpo.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, el cual es un Nanocuerpo humanizado.

y algunos aspectos preferidos de esta modalidad se refieren a:

- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, el cual es un Nanocuerpo humanizado clase GLEW.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, el cual es un Nanocuerpo humanizado clase GLEW.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, el cual contiene un residuo de arginina (R) en la posición 103.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, el cual contiene un residuo de

669

arginina (R) en la posición 103, y el cual es humanizado.

- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, el cual es un Nanocuerpo de clase GLEW, y el cual contiene un residuo de arginina (R) en la posición 103, y el cual es humanizado.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, el cual contiene un residuo de Lisina (L) en la posición 108.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, el cual tiene al menos, 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en este documento) con una de las secuencias de aminoácido de la SEC ID NO 52 (TNF1), 76 (TNF13), 77 (TNF14), 95 (TNF29) ó 96 (TNF30).
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en el cual

a) CDR1 comprende:

- la secuencia de aminoácido DYWMY; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido

670

DYWMY; o

- una secuencia de aminoácido que tiene 2 o solamente 1 diferencia(s) de aminoácido con la secuencia de aminoácido DYWMY;

y

b) CDR2 comprende:

- la secuencia de aminoácido EINTNGLITKYPDSVKG;

- o una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido EINTNGLITKYPDSVKG; o

- una secuencia de aminoácido que tiene 2 o solamente 1 diferencia(s) de aminoácido con la secuencia de aminoácido EINTNGLITKYPDSVKG;

y

c) CDR3 comprende:

- la secuencia de aminoácido SPSGFN; ó

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido SPSGFN; o una secuencia de aminoácido que tiene 2 o solamente 1 diferencia(s) de aminoácido con la secuencia de aminoácido SPSGFN.

671

- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales CDR1 comprende la secuencia de aminoácido DYWMY.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales CDR3 comprende la secuencia de aminoácido SPSGFN.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales:
  - CDR1 comprende la secuencia de aminoácido DYWMY; y CDR3 comprende la secuencia de aminoácido SPSGFN; o
  - CDR1 comprende la secuencia de aminoácido DYWMY; y CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG; o
  - CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG; y CDR3 comprende la secuencia de aminoácido SPSGFN.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales CDR1 comprende la secuencia de aminoácido DYWMY; y CDR3 comprende la secuencia de aminoácido SPSGFN.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales CDR1 comprende la

672

secuencia de aminoácido DYWMY; CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG y CDR3 comprende la secuencia de aminoácido SPSGFN.

- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales

a) CDR1 es:

- la secuencia de aminoácido DYWMY; o

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido DYWMY; o

- una secuencia de aminoácido que tiene 2 o solamente 1 diferencia de aminoácido con la secuencia de aminoácido DYWMY;

y en los cuales:

b) CDR2 es:

- la secuencia de aminoácido EINTNGLITKYPDSVKG; o

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido EINTNGLITKYPDSVKG; o

- una secuencia de aminoácido que tiene 2 o solamente 1 diferencia(s) de aminoácido con la secuencia de

aminoácido EINTNGLITKYPDSVKG;

y en los cuales

c) CDR3 es:

- la secuencia de aminoácido SPSGFN; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido SPSGFN; o
- una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia de aminoácido con la secuencia de aminoácido SPSGFN.

- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales CDR1 es la secuencia de aminoácido DYWMY.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales CDR2 es la secuencia de aminoácido EINTNGLITKYPDSVKG.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales CDR3 es la secuencia de aminoácido SPSGFN.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales:
  - CDR1 es la secuencia de aminoácido DYWMY; y CDR3 es la secuencia de aminoácido SPSGFN; o

- CDR1 es la secuencia de aminoácido DYWMY; y CDR2 es la secuencia de aminoácido EINTNGLITKYPDSVKG; o

- CDR2 es la secuencia de aminoácido EINTNGLITKYPDSVKG; y CDR3 es la secuencia de aminoácido SPSGFN.

- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales CDR1 es la secuencia de aminoácido DYWMY; y CDR3 es la secuencia de aminoácido SPSGFN.

- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales CDR1 es la secuencia de aminoácido DYWMY; CDR2 es la secuencia de aminoácido EINTNGLITKYPDSVKG y CDR3 es la secuencia de aminoácido SPSGFN.

- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales

- cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativo; y/o dicha secuencia de aminoácido preferiblemente contiene solamente sustituciones de aminoácido, y no supresiones o inserciones de aminoácido, comparadas con la(s) secuencia(s) de aminoácido(s) anteriores,

y algunos otros aspectos preferidos de esta modalidad que se refieren a:

- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, el cual es un Nanocuerpo clase KERE.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, el cual es un Nanocuerpo clase KERE humanizado
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, el cual tiene al menos, 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en este documento) con una de las secuencias de aminoácido de las SEC ID NOs. 50 (TNF3), 83 (TNF20), 85 (TNF21), 85 (TNF22), 96 (TNF23) ó 98 (TNF33).
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales

a) CDR1 es:

- la secuencia de aminoácido NYYMG; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido NYYMG; o
- una secuencia de aminoácido que tiene 2 o solamente 1 diferencia de aminoácido con la secuencia de

aminoácido NYYMG;

y

b) CDR2 es:

- la secuencia de aminoácido NISWRGYNIYYKDSVKG; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido NISWRGYNIYYKDSVKG; o
- una secuencia de aminoácido que tiene 2 o solamente 1 diferencia(s) de aminoácido con la secuencia de aminoácido NISWRGYNIYYKDSVKG;

y c) CDR3 es:

- la secuencia de aminoácido SILPLSDDPGWNTY; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido SILPLSDDPGWNTY; o
- una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia de aminoácido con la secuencia de aminoácido SILPLSDDPGWNTY.

- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales CDR1 es la secuencia de aminoácido NYYMG.

- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales CDR2 es la secuencia de aminoácido NISWRGYNIYYKDSVKG.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales CDR3 es la secuencia de aminoácido SILPLSDDPGWNTY.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales:
  - CDR1 es la secuencia de aminoácido NYYMG; y CDR3 es la secuencia de aminoácido SILPLSDDPGWNTY; o
  - CDR1 es la secuencia de aminoácido NYYMG; y CDR2 es la secuencia de aminoácido NISWRGYNIYYKDSVKG; o
  - CDR2 es la secuencia de aminoácido NISWRGYNIYYKDSVKG; y CDR3 es la secuencia de aminoácido SILPLSDDPGWNTY.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales CDR1 es la secuencia de aminoácido NYYMG; CDR2 es la secuencia de aminoácido SILPLSDDPGWNTY y CDR3 es la secuencia de aminoácido ILPLSDDPGWNTY.
- Un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores, en los cuales
  - cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativo; y/o

- dicha secuencia de aminoácido preferiblemente contiene, solamente sustituciones de aminoácido, y no supresiones o inserciones de aminoácido, comparadas con la(s) secuencia(s) de aminoácido(s) anteriores.

y con aún algunos otros aspectos particularmente preferidos siendo:

VI) Una proteína o polipéptido, el cual comprende un o consiste esencialmente de un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores.

VII) Una proteína o polipéptido, el cual comprende o consiste esencialmente de al menos un Nanocuerpo de conformidad con cualquiera de I) hasta V) anteriores.

VIII) Una proteína o polipéptido, el cual comprende dos Nanocuerpos de conformidad con cualquiera de I) hasta V) anteriores.

IX) Una proteína o polipéptido, el cual comprende dos Nanocuerpos de conformidad con cualquiera de I) hasta V) anteriores, y el cual es tal que dicha proteína o polipéptido, después de enlazarse a un trímero de TNF, es capaz de inhibir o reducir la reticulación del receptor de TNF que es mediada por dicho trímero de TNF y/o la transducción de señal que es mediada por tal reticulación de receptor.

X) Una proteína o polipéptido, el cual comprende dos Nanocuerpos de conformidad con cualquiera de I) hasta

V) anteriores, y el cual es capaz de enlazarse intramolecularmente con al menos, dos sitios de enlace del receptor de TNF en un trímero de TNF.

XI) Una proteína o polipéptido, el cual comprende dos Nanocuerpos de conformidad con cualquiera de I) hasta V) anteriores, enlazados vía un enlazador adecuado.

XII) Una proteína o polipéptido, el cual comprende dos Nanocuerpos de conformidad con cualquiera de I) hasta V) anteriores, enlazados vía un enlazador adecuado, y el cual es pegilado.

XIII) Una proteína o polipéptido el cual comprende dos Nanocuerpos de conformidad con cualquiera de I) hasta V) anteriores, y el cual además, comprende al menos un Nanocuerpo dirigido contra albúmina de suero humana.

XIV) Una proteína o polipéptido el cual comprende dos Nanocuerpos de conformidad con cualquiera de I) hasta V) anteriores, y el cual además comprende al menos un Nanocuerpo dirigido contra albúmina de suero humana, y el cual es tal que dicha proteína o polipéptido, después de enlazarse a un trímero de TNF, es capaz de inhibir o reducir la reticulación de receptor de TNF que es mediada por dicho trímero de TNF y/o la transducción de señal que es mediada por tal reticulación de receptor.

XV) Una proteína o polipéptido el cual comprende dos

Nanocuerpos de conformidad con cualquiera de I) hasta V) anteriores, y el cual además comprende al menos un Nanocuerpo dirigido contra albúmina de suero humana y el cual es capaz de enlazarse intramolecularmente con al menos, dos sitios de enlace del receptor de TNF en un trímero de TNF.

XVI) Una proteína o polipéptido el cual comprende dos Nanocuerpos de conformidad con cualquiera de I) hasta V) anteriores, y el cual además comprende un Nanocuerpo dirigido contra albúmina de suero humana, en los cuales cada uno de los dos Nanocuerpos de conformidad con cualquiera de I) hasta V) anteriores es enlazado, opcionalmente vía un enlazador adecuado, a un Nanocuerpo dirigido contra albúmina de suero humana.

XVII) Una proteína o polipéptido el cual comprende dos Nanocuerpos de conformidad con cualquiera de I) hasta V) anteriores, y el cual además comprende un Nanocuerpo dirigido contra albúmina de suero humana, en los cuales cada uno de los dos Nanocuerpos de conformidad con cualquiera de I) hasta V) anteriores es enlazado, opcionalmente vía un enlazador adecuado, a un Nanocuerpo dirigido contra albúmina de suero humana, y el cual es tal que dicha proteína o polipéptido, después de enlazarse a un trímero de TNF,

es capaz de enlazarse o reducir la reticulación del receptor de TNF que es mediada por dicho trímero de TNF y/o la transducción de señal que es mediada por tal reticulación del receptor.

XVIII) Una proteína o polipéptido el cual comprende dos Nanocuerpos de conformidad con cualquiera de I) hasta V) anteriores, y el cual además comprende un Nanocuerpo dirigido contra albúmina de suero humana, en los cuales cada uno de los dos Nanocuerpos de conformidad con cualquiera de I) hasta V) anteriores es enlazado, opcionalmente vía un enlazador adecuado, a un Nanocuerpo dirigido contra albúmina de suero humana, y el cual es capaz de enlazarse intramolecularmente con al menos dos sitios de enlace del receptor de TNF en un trímero de TNF.

- Una proteína o polipéptido de conformidad con cualquiera de VI) hasta XVIII) anteriores, en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es un Nanocuerpo humanizado.
- Una proteína o polipéptido de conformidad con cualquiera de VI) hasta XVIII) anteriores, en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es una variante humanizada del Nanocuerpo ALB 1 (SEC ID NO: 63).
- Una proteína o polipéptido de conformidad con

cualquiera de VI) hasta XVIII) anteriores, en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es uno elegido del grupo que consiste de ALB 3 (SEC ID NO: 87), ALB 4 (SEC ID NO: 88), ALB 5 (SEC ID NO: 89), ALB 6 (SEC ID NO: 100), ALB 7 (SEC ID NO: 101), ALB 8 (SEC ID NO: 102) ALB 9 (SEC ID NO: 103) y ALB 10 (SEC ID NO: 104).

- Una proteína o polipéptido de conformidad con cualquiera de VI) hasta XVIII) anteriores, en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es ALB 8.
- Una proteína o polipéptido de conformidad con cualquiera de VI) hasta XVIII) anteriores, el cual comprende o consiste esencialmente de dos Nanocuerpos humanizados de conformidad con cualquiera de I) hasta V) anteriores, y una variante humanizada del Nanocuerpo ALB 1 (SEC ID NO: 63).

Se debe notar que cuando un Nanocuerpo es mencionado anteriormente por estar "*de conformidad con cualquiera de I) a V) anteriores*", es al menos, de conformidad con uno de I) a V), pueden estar de conformidad con dos o más de I) a V), y puede también, incluir cualquiera o más de los otros aspectos que están indicados por estar "de conformidad con cualquiera de I) a V) anteriores. De manera similar, cuando una proteína o

polipéptido es mencionado anteriormente por estar *"de conformidad con cualquiera de VI) a XVIII) anteriores"*, está al menos de conformidad con uno de VI) a XVIII), puede ser de conformidad con dos o más de VI) a XVIII), y puede también incluir cualquiera o más de los otros aspectos que están indicados por estar "de conformidad con cualquiera de VI) a XVIII) anteriores.

Está también dentro del alcance de la invención que, en donde sea aplicable, un Nanocuerpo o polipéptido de la invención, puede enlazarse a dos o más determinantes antigénicas, epítopes, partes, dominios, subunidades o confirmaciones de TNF-alfa. En tal caso, las determinantes antigénicas, epítopes, partes, dominios o subunidades de TNF-alfa a las cuales los Nanocuerpos y/o polipéptidos de la invención se enlazan, pueden ser esencialmente el mismo (por ejemplo, si el TNF-alfa contiene porciones estructurales repetidas o está presente como un multímero), o puede ser diferente (y en el último caso, los Nanocuerpos o polipéptidos de la invención pueden enlazarse a tales determinantes antigénicas diferentes, epítopes, partes, dominios, subunidades de TNF-alfa con una afinidad y/o especificidad, la cual puede ser la misma o diferente). También, por ejemplo, cuando existe TNF-alfa en una conformación activada y en una conformación inactiva, los Nanocuerpos y polipéptidos de la invención, pueden

enlazarse a ya sea una de estas conformaciones, o puede enlazarse a ambas de estas conformaciones (es decir, con una afinidad y/o especificidad la cual puede ser la misma o diferente). También, por ejemplo, los Nanocuerpos y polipéptidos de la invención pueden enlazarse a una conformación de TNF-alfa en la cual, se unen a un ligando pertinente, pueden enlazarse a una conformación de TNF-alfa en la cual, no se unen a un ligando pertinente, o pueden enlazarse a ambas de tales conformaciones (nuevamente con una afinidad y/o especificidad la cual puede ser la misma o diferente).

También, se espera que los Nanocuerpos y polipéptidos de la invención, en general, se enlazarán a todos los análogos, variantes, mutantes alelos, partes y fragmentos de TNF-alfa, sintéticos o que se originan naturalmente, o al menos, a aquellos análogos, variantes, mutantes, alelos, partes y fragmentos de TNF-alfa que contienen una o más determinantes antigénicas o epítopes que son esencialmente los mismos con el(los) determinante(s) o epítope(s) antigénicos a los cuales los Nanocuerpos y polipéptidos de la invención se enlaza en TNF-alfa (por ejemplo, en TNF-alfa de tipo nativo). Nuevamente, en tal caso, los Nanocuerpos y polipéptidos de la invención pueden enlazarse a tales análogos, variantes, mutantes, alelos, partes y fragmentos con una afinidad y/o

especificidad de que son los mismos como, o que difieren de (es decir, superior que o inferior que), la afinidad y especificidad con la cual los Nanocuerpos de la invención se enlazan a TNF-alfa (de tipo nativo). También se incluyen dentro del alcance de la invención, que los Nanocuerpos y polipéptidos de la invención, se enlazan a algunos análogos, variantes, mutantes, alelos, partes y fragmentos de TNF-alfa, pero no a otros.

En general, los Nanocuerpos y polipéptidos de la invención al menos, se enlazarán a aquellas formas (que incluyen formas monoméricas, multiméricas y asociadas), que son las más relevantes de un punto de vista biológico y/o terapéutico, como será claro para la persona experta.

También, como el TNF-alfa existe en una forma monomérica y en formas multiméricas, y en particular, en formas triméricas, está dentro del alcance de la invención, que los Nanocuerpos y polipéptidos de la invención solamente se enlazan a TNF-alfa en forma monomérica, o que los Nanocuerpos y polipéptidos de la invención, además de enlazarse también a una o más de tales formas multiméricas, tales como la forma trimérica de TNF; o pueden solamente enlazarse a tal forma multimérica (por ejemplo trimérico). De este modo, en general, cuando en esta descripción se hace referencia a un Nanocuerpo, proteína o polipéptido que es dirigido contra TNF-alfa, se debe entender que este

también comprende Nanocuerpos dirigidos contra el TNF-alfa en su forma trimérica (que incluye pero no se limita a, Nanocuerpos contra los sitios de enlace al receptor (por ejemplo, los sitios de enlace para TNF-RI, THF-RII, también conocido como p55 ó p75) de tal trímero). En todos estos casos, los Nanocuerpos y polipéptidos de la invención se pueden enlazar a tales multímeros o complejos de proteína asociada con una afinidad y/o especificidad que pueden ser el mismo o diferentes de (es decir, superior o inferior), que la afinidad y/o especificidad con la cual, los Nanocuerpos y polipéptidos de la invención se enlazan a TNF-alfa en su estado monomérico y no asociado.

También, en general, los polipéptidos de la invención que contienen dos o más Nanocuerpos dirigidos contra TNF-alfa, pueden enlazarse con avidez superior que los Nanocuerpos o Nanocuerpo monomérico correspondiente.

Por ejemplo, y sin limitación, una proteína multivalente (como se define en este documento) o polipéptido que contiene dos o más Nanocuerpos que se dirigen contra diferentes epítopes de proteína o polipéptido multivalente TNF-alfa (como se define en este documento), que contiene dos o más Nanocuerpos que se dirigen contra epítopes diferentes de TNF-alfa, pueden enlazarse a TNF-alfa con avidez superior que los monómeros correspondientes.

De manera más importante, una proteína o polipéptido multivalente (como se define en este documento), que contiene dos o más Nanocuerpos que se dirigen contra TNF-alfa (y usualmente) se enlazarán con avidez superior a un multímero de TNF-alfa que a un monómero de TNF-ala, y usualmente también se enlazarán con avidez superior que los Nanocuerpos monoméricos correspondientes. En tal proteína o polipéptido multivalente, los dos o más Nanocuerpos pueden por ejemplo, ser dirigidos contra los mismos epítopes, sustancialmente epítopes equivalentes, o diferentes epítopes. En una modalidad de tal proteína o polipéptido multivalente, los dos o más Nanocuerpos pueden ser los mismos (y por lo tanto, ser dirigidos contra el mismo epítope).

El último es de particular importancia, como se sabe que el modo primario de la transducción de señal por TNF involucra reticulación por receptores de TNF por un trímero de moléculas de TNF, los cuales contienen tres sitios de enlace al receptor (véase por ejemplo, Peppel et al., *J. Exp. Med.*, 174 (1991), 1483-1489; Engelmann et al., *J. Biol. Chem.*, 265 (1990), 14497; Smith and Baglioni, J. *Biol. Chem.*, 264 (1989), 14646). Por ejemplo, como se describe por Peppel et al., un dominio extracelular monovalente diseñado por ingeniería del receptor de TNF- el cual fue solamente capaz de bloquear un sitio de enlace al

receptor único en un trímero de TNFa, -fue incapaz de prevenir la reticulación de los receptores de TNF por los restantes dos sitios de enlace al receptor; mientras una proteína diseñada por ingeniería que comprende dos de tales dominios extracelulares - de este modo siendo capaz de bloquear dos sitios de enlace al receptor -proporciona una eficacia sorprendente, comparada con el dominio extracelular monovalente.

En la presente invención, se ha encontrado que los Nanocuerpos monovalentes, son capaces de enlazarse a TNF-alfa en tal forma que, la actividad del TNF se reduce, tanto en modelos in vitro, en modelos celulares como en modelos ex vivo (véase la Sección Experimental abajo). Aunque la invención no está limitada a cualquier mecanismo, explicación o hipótesis específica, se asume que debido a su tamaño más pequeño y alta afinidad para TNF-alfa, dos o tres Nanocuerpos monovalentes de la invención, son capaces de ocupar simultáneamente dos o tres diferentes sitios de enlace al receptor en el trímero de TNF, de este modo, previniendo al trímero de iniciar la reticulación del receptor y con ello, iniciar la transducción de la señal (sin embargo, otros mecanismos de acción no son excluidos: por ejemplo, dependiendo del epítope contra el cual se dirige, un Nanocuerpo de la invención también puede inhibir la asociación de TNF en el estado trimérico).

Se debe notar también que, además o como una alternativa para enlazarse a dos o más sitios de enlace al receptor en un trímero de TNF-único, las proteínas o polipéptidos de la presente invención que comprenden o esencialmente consisten de dos o más dominios variables de inmunoglobulina (o fragmentos adecuados de los mismos), que son dirigidos contra epítopes de TNF-alfa, pueden enlazar epítopes (por ejemplo, intramolecularmente), en dos moléculas de TNF-alfa separadas (por ejemplo, dos trímeros separados).

Sin embargo, de conformidad con una modalidad particularmente preferida, la invención se refiere a una proteína o polipéptido que comprende o esencialmente consiste de dos o más dominios variables de inmunoglobulina (o fragmentos adecuados de los mismos), que son cada uno dirigidos contra epítopes en TNF-alfa (y en particular, del trímero de TNF-alfa), que caen en y/o forman parte del(los) sitio(s) de enlace(s) del receptor del trímero de TNF, de manera tal que dicho polipéptido, después de enlazarse a un trímero de TNF, es capaz de inhibir o reducir la reticulación del receptor de TNF que es mediada por dicho trímero de TNF y/o la transducción de señal que es mediada por tal reticulación del receptor.

En particular, de conformidad con esta modalidad preferida, la invención se refiere a un polipéptido de

proteína que comprende o esencialmente consiste de dos o más dominios variables de inmunoglobulinas (o fragmentos adecuados de los mismos), que son cada uno, dirigidos contra epítopes en TNF-alfa (y en particular, del trímero TNF-alfa), que caen en y/o forman parte del(los) sitio(s) de enlace al receptor del trímero de TNF, en donde dichos dominios variables de inmunoglobulina son ligados entre sí en tal forma que la proteína o polipéptido, son capaces de enlazarse simultáneamente a dos o más sitios de enlace al receptor en un trímero de TNF único (en otras palabras, es capaz de enlazarse intramolecularmente con al menos, dos sitios de enlace al receptor de TNF en un trímero de TNF). En esta modalidad, los dos o más dominios variables de inmunoglobulina, son preferiblemente como se definen anteriormente y son más preferiblemente, Nanocuerpos (ya que la proteína o polipéptido es un constructo de Nanocuerpo multivalente, como se describe en este documento adicionalmente). También, en esta modalidad, los dos o más dominios variables de inmunoglobulinas, pueden ser los mismos o diferentes; y pueden ser dirigidos contra epítopes diferentes con el(los) sitio(s) de enlace al receptor de TNF, pero son preferiblemente, dirigidos contra el mismo epítope.

En un aspecto preferido de esta modalidad, los dos o más dominios variables de inmunoglobulina, son

dirigidos contra epítopes del trímero de TNF-alfa, en el cual, los epítopes caen y/o forman parte del(los) sitios de enlace del receptor de TNF. Por ejemplo, los dos o más dominios variables de inmunoglobulina son preferiblemente, dirigidos contra y/o pueden enlazarse a un epítope del trímero TNF-alfa que comprende los siguientes residuos de aminoácido: Gln en la posición 88 y Lys en la posición 90 en un primer monómero de TNF (referido en este documento como "monómero A"), y Glu en la posición 146 en un segundo monómero de TNF (referido aquí como "monómero B") (en el cual, el Monómero A y el Monómero B en conjunto, en el trímero de TNF, forman el(los) sitio(s) de enlace al receptor de TNF.

Como se describe además abajo en más detalles con respecto a Nanocuerpos, en tal proteína o polipéptido, al menos, dos dominios variables de inmunoglobulina son preferiblemente ligados en tal forma que la distancia entre el N-término y el C-término de los dos dominios variables de inmunoglobulina, presentes en tal proteína o polipéptido, son preferiblemente al menos, 50 Angstroms, y más preferiblemente, en la región de 55-200 Angstroms, y más preferiblemente en la región de Angstroms, y en particular en la región de 65-150 Angstroms.

En un aspecto particularmente preferido de esta modalidad, estas dos o más secuencias de inmunoglobulina

son Nanocuerpos y son preferiblemente elegidos a partir de los Nanocuerpos descritos en este documento. Algunos Nanocuerpos particularmente preferidos para uso en esta modalidad de la invención son PMP1C2 (TNF1, SEC ID NO:52) y/o PMP5F10 (TNF3, SEC ID NO:60), así como también variantes humanizadas y otras de las mismas (como se describe en este documento); con PMP1C2 (TNF1, SEC ID NO:52) y sus variantes humanizadas siendo particularmente preferidas.

Por consiguiente, la presente modalidad será ahora descrita en más detalle con referencia a Nanocuerpos. Sin embargo, será claro para la persona experta, que las enseñanzas de este documento, pueden ser aplicadas análogamente a dominios variables de inmunoglobulinas.

En esta modalidad de la invención, las dos o más secuencias de inmunoglobulina usualmente, serán ligadas vía uno o más enlazadores adecuados, en los cuales, los enlazadores son tal que cada secuencia de inmunoglobulina puede enlazarse a un sitio de enlace al receptor diferente en el mismo trímero de TNF. Los enlazadores adecuados dependerán entre otras cosas, de (la distancia entre) los epítopes en el trímero de TNF en el cual las secuencias de inmunoglobulina se enlazan, y será claro para la persona experta basada en la descripción de este documento, opcionalmente después de algún grado limitado de

experimentación de rutina. Por ejemplo, cuando dos o más secuencias de inmunoglobulina son anticuerpos o Nanocuerpos de dominio (único), enlazadores adecuados pueden ser elegidos a partir de los enlazadores descritos en este documento, pero con una longitud de enlazador que es tal que los dos o más anticuerpos o Nanocuerpos de dominio (único), pueden cada uno, enlazarse a un sitio de enlace al receptor diferente en el mismo trímero.

También, cuando dos o más secuencias de inmunoglobulina que se enlazan a los sitios de enlace al receptor de TNF-alfa son anticuerpos o Nanocuerpos de domino (único), pueden también ser ligados entre sí, vía un tercer anticuerpo o Nanocuerpo de dominio (único) (en el cual, las dos o más secuencias de inmunoglobulina pueden ser ligadas directamente al tercer anticuerpo/Nanocuerpo de dominio (único) o vía enlazadores adecuados). Tal tercer anticuerpo o Nanocuerpo de dominio (único) puede por ejemplo, ser un anticuerpo o Nanocuerpo de dominio (único) que se proporciona para una vida media incrementada, como se describe además, en este documento. Por ejemplo, el anticuerpo o Nanocuerpo de dominio tardío (único), puede ser un anticuerpo o Nanocuerpo de dominio (único) que es capaz de enlazarse a una proteína del suero (humana), tal como albúmina de suero (humana), como se describe además posteriormente.

Alternativamente, dos o más de las secuencias de inmunoglobulina que se enlazan al(los) sitios de enlace al receptor de TNF-alfa, pueden ser ligadas en serie (ya sea directamente o vía un enlazador adecuado) y el tercer anticuerpo o Nanocuerpo de dominio (único), (el cual puede proporcionar una vida media incrementada, como se describe anteriormente), puede ser conectado directamente o vía un enlazador a una de estas dos o más secuencias de inmunoglobulina mencionadas anteriormente. Algunos ejemplos no limitantes de tales constructos son los constructos de las SEC ID Nos: 93 ó 94.

En particular, se ha encontrado en la invención (véase los datos de cristalografía referidos en este documento), que cuando los Nanocuerpos presentes en una proteína o polipéptido multivalente o multiespecífico de la invención se enlazan al epítope particular descrito anteriormente (el cual es el epítope de TNF1 y sus variantes humanizadas, así como también del TNF3 y sus variantes humanizadas), entonces, preferiblemente, los dos (o más) anticuerpos anti-TNF presentes en tal proteína o polipéptido, deben ser enlazados en tal forma que la distancia entre el N-término y el C-término de los dos Nanocuerpos anti-TNF presentes en tal proteína o polipéptido, deben ser preferiblemente, al menos de 50 Angstroms, y más preferiblemente, en la región de 55-200

Angstroms, y en particular, en la región de 65-150 Angstroms (con el límite superior siendo menos crítico, y siendo elegido por razones de conveniencia, por ejemplo con una vista a la expresión/producción de la proteína); o más en general, que dicha distancia debe ser tal que permite la proteína o polipéptido para someterse al enlace intramolecular al trímero de TNF (es decir, en lugar del enlace intramolecular). La distancia entre el N-término y el C-término de dos Nanocuerpos anti-TNF, puede ser determinada por cualquiera de los medios adecuados, tal como por cristalografía o modelado molecular (como se describe en este documento). Estas técnicas en general, también hacen posible determinar si una proteína o polipéptido multivalente específico o multiespecífico, es capaz de proporcionar modelado intramolecular. Alternativamente, la presente invención también proporciona un experimento simple usando cristalografía por exclusión de tamaño (como se describe por Santora et al., Anal. Biochem., 299: 119-129), que puede ser usada para determinar si una proteína o polipéptido dado de la invención, proporcionará (predominantemente) el enlace intramolecular a un trímero de TNF o (predominantemente) el enlace intramolecular entre dos o más trímeros de TNF. De este modo, en una modalidad particular de la invención, una proteína o polipéptido de la invención es preferiblemente

de manera tal que en este experimento, predominantemente o esencialmente, exclusivamente conduce a enlace intramolecular. Sin embargo, como se enfatiza anteriormente, se debe notar que las proteínas o polipéptidos de la invención que operan vía enlace intramolecular de moléculas de TNF-alfa separadas (por ejemplo, trímeros), también están dentro del alcance de la presente invención.

De este modo, en otro aspecto preferido, la invención proporciona una proteína o polipéptido multivalente o multiespecífico que comprende al menos, dos Nanocuerpos contra TNF-alfa (y en particular, del trímero de TNF-alfa), en el cual dichos Nanocuerpos son preferiblemente dirigidos a esencialmente el mismo epítope como el Nanobody PMP1C2 (como se mencionó en este documento), y en el cual, al menos dos Nanocuerpos están ligados en tal forma que la distancia entre el N-término y el C-término de al menos, dos Nanocuerpos anti-TNF es tal, que la proteína o polipéptido es capaz de someterse a enlace intramolecular (como se describe aquí), con un trímero de TNF. Preferiblemente, en tal proteína o polipéptido, la distancia entre el N-término y el C-término de dos Nanocuerpos anti-TNF, es al menos, 50 Angstroms, y más preferiblemente, en la región de 55-200 Angstroms, y en particular, en la región de 65-150 Angstroms.

En tal polipéptido o proteína preferidas, los dos o más Nanocuerpos pueden ser ligados en cualquier forma adecuada, tan pronto como la distancia preferida entre el N-término y el C-término de al menos dos Nanocuerpos anti-TNF se pueda lograr, y/o tan pronto como la proteína o polipéptido sea capaz de someterse a enlace intramolecular (como se describe en este documento), con un trímero de TNF.

Por ejemplo, en su forma más simple, al menos dos Nanobodies son directamente ligados vía un enlazador o espaciador adecuado que proporciona la distancia preferida entre el N-término y el C-término de al menos, dos Nanocuerpos anti-TNF y el cual puede permitir a la proteína o polipéptido, someterse a enlace intramolecular (como se describe en este documento), con un trímero de TNF. Los enlazadores adecuados son descritos en este documento, y pueden - por ejemplo y sin limitación -, comprender una secuencia de aminoácido, en la cual, la secuencia de aminoácido preferiblemente tiene una longitud de 14 aminoácidos, más preferiblemente, al menos 17 aminoácidos, tal como una secuencia de aproximadamente 20-40 aminoácidos (en la cual, usando una distancia promedio de 3.5 Angstrom por un aminoácido, corresponde a las longitudes de enlazador de 49 Angstrom, 59.5 Angstroms y aproximadamente 70 Angstroms, respectivamente; con la cantidad máxima de

aminoácidos siendo calculada en la misma forma, basada en las distancias mencionadas anteriormente). Preferiblemente, tal secuencia de aminoácido también debe ser tal que permita a la proteína o polipéptido, someterse a enlace intramolecular (como se describe en este documento), con un trímero de TNF.

De este modo, en otro aspecto preferido, la invención proporciona una proteína o polipéptido multivalente o multiespecífico que comprende, al menos, dos Nanocuerpos contra TNF-alfa (y en particular, del trímero TNF-alfa), en el cual, dichos Nanocuerpos son preferiblemente dirigidos a esencialmente el mismo epítope como Nanocuerpo PMP1C2 (como se menciona en este documento), y en el cual, al menos dos Nanocuerpos son directamente ligados entre sí, usando un enlazador o espaciador adecuado, de manera tal que la distancia entre el N-término y el C-término de al menos dos Nanocuerpos anti-TNF es tal, que la proteína o polipéptido es capaz de someterse a enlace intramolecular (como se describe en este documento), con un trímero de TNF. Preferiblemente, en tal proteína o polipéptido, la distancia entre el N-término y el C-término de dos Nanocuerpos anti-TNF (y con ello, la longitud preferida del enlazador o espaciador) es al menos, 50 Angstroms, y más preferiblemente en la región de 55-200 Angstroms, y en particular, en la región de 65-150

Angstroms.

Más preferiblemente, en este aspecto preferido, el enlazador o espaciador es una secuencia de aminoácido que comprende al menos 14, preferiblemente al menos 17, más preferiblemente al menso 20 aminoácidos (con un límite superior no crítico elegido por razones de conveniencia, siendo aproximadamente 50, y preferiblemente aproximadamente 40 aminoácidos). En una modalidad preferida, pero no limitantes, el enlazador esencialmente consiste de residuos glicina y serina (como se describe además posteriormente). Por ejemplo, un enlazador adecuado es el enlazador GS30 descrito en este documento, el cual comprende 30 residuos de aminoácido.

En otra modalidad, al menos dos Nanocuerpos contra TNF-alfa son ligados entre sí, vía otra porción (opcionalmente vía uno o dos enlazadores), tal como otra proteína o polipéptido. En esta modalidad, puede ser deseable tener la distancia preferida (es decir, como se menciona anteriormente), entre el N-término y el C-término de al menos, dos Nanocuerpos anti-TNF, por ejemplo, de manera tal que la proteína o polipéptido puede todavía someterse a enlace intramolecular (como se describe en este documento), con un trímero de TNF. En esta modalidad, al menos dos Nanocuerpos pueden ser ligados directamente a otra porción, o usando un enlazador o espaciador adecuado,

700

nuevamente, tan pronto como la distancia preferida y/o enlace intramolecular deseado, pueda todavía lograrse. La porción puede ser cualquier porción adecuada la cual no se detracte (lo suficiente), del enlace de la proteína o polipéptido al TNF y/o de las propiedades farmacológicas o biológicas deseadas de la proteína o polipéptido. Como tal, la porción puede ser esencialmente inactiva o puede ser biológicamente activa, y como tal, puede o no puede mejorar las propiedades deseadas de la proteína o polipéptido y/o puede conferir una o más propiedades deseadas adicionales a la proteína o polipéptido. Por ejemplo, y sin limitación, la porción puede mejorar la vida media de la proteína o polipéptido, y/o puede reducir su inmunogenicidad o mejorar cualquier otra propiedad deseada.

En una modalidad preferida, la porción puede ser otro Nanocuerpo (que incluye pero no se limita al tercer Nanocuerpo contra TNF-alfa, aunque esto no es necesario y usualmente menos preferido), y en particular otro Nanocuerpo que mejora la vida media de la proteína o polipéptido, tal como un Nanocuerpo que es dirigido contra una proteína del suero, por ejemplo, contra albúmina de suero humano. Ejemplos de tales proteínas y polipéptidos son descritos en este documento.

De este modo, en una modalidad, la invención se refiere a un constructo multiespecífico multivalente que

comprende, dos o más secuencias de inmunoglobulina (o fragmentos adecuados de los mismos), que son cada uno, dirigidos contra epítopes en TNF-alfa (por ejemplo del trímero TNF-alfa) que cae en y/o forma parte del sitio de enlace al receptor, y que están ligados entre sí, vía al menos, una secuencia de inmunoglobulina que proporciona vida media incrementada (y opcionalmente, vía uno o más enlazadores adecuados), de manera tal que dicho polipéptido, después del enlace a un trímero de TNF, es capaz de inhibir o reducir la reticulación del receptor de TNF y/o la transducción de la señal que es mediada por dicho trímero de TNF. Tal polipéptido puede ser tal que es dichas dos o más secuencias de inmunoglobulina mencionadas anteriormente, pueden cada una, enlazarse a un sitio de enlace al receptor diferente en un trímero de TNF.

En particular, en esta modalidad, el polipéptido puede comprender un Nanocuerpo biespecífico trivalente, que comprende dos Nanocuerpos que son cada uno, dirigidos contra epítopes en TNF-alfa (y en particular, del trímero de TNF-alfa), que caen en y/o forman parte del sitio de enlace al receptor, en el cual, dichos Nanocuerpos están ligados entre sí vía un tercer Nanocuerpo que proporciona una vida media incrementada (por ejemplo, un Nanocuerpo que se dirige a una proteína del suero tal como albúmina de suero humano), en el cual, cada uno de los dos Nanocuerpos

mencionados primero, puede ser directamente ligado a dicho tercer Nanocuerpo o vía uno o más enlazadores adecuados, de manera tal que dicho polipéptido después del enlace a un trímero de TNF, es capaz de inhibir o reducir la reticulación del receptor de TNF y/o la transducción de señal que es mediada por dicho trímero de TNF. Tal polipéptido puede ser de manera que dichos dos Nanocuerpos mencionados primero, pueden cada uno, enlazarse a un sitio de enlace al receptor diferente en un trímero de TNF. Nuevamente, algunos Nanocuerpos particularmente preferidos para uso en esta modalidad de la invención, son PMP1C2 (TNF1, SEC ID NO:52) y/o PMP5F10 (TNF3, SEC ID NO:60), así como también variantes humanizadas y otras de los mismos (como se describe en este documento); con PMP1C2 (TNF1, SEC ID NO:52) y sus variantes humanizadas, son particularmente preferidos; y los Nanocuerpos dirigidos contra albúmina de suero humano descrito en este documento. Algunos constructos preferidos, pero no limitantes de esta modalidad de la invención son, TNF24 (SEC ID NO:90), TNF 26 (SEC ID NO:92), TNF 27 (SEC ID NO:93), TNF 28 (SEC ID NO:94), TNF 60 (SEC ID NO:417) y TNF 62 (SEC ID NO:418), de los cuales TNF 60 es particularmente preferido.

De este modo, algunos aspectos preferidos de esta modalidad de la invención se refieren a:

XIX) Una proteína o polipéptido que comprende o consiste

esencialmente, de dos o más dominios variables de inmunoglobulina (o fragmentos adecuados de los mismos) que son cada uno dirigidos contra epítopes en TNF-alfa (y en particular, del trímero TNF-alfa) que cae y/o forma parte del(los) sitio(s) de enlace al receptor del trímero de TNF, de manera tal que dicho polipéptido, después del enlace a un trímero de TNF, es capaz de inhibir o reducir la reticulación del receptor de TNF que es mediada por dicho trímero de TNF y/o la transducción de señal que es mediada por tal reticulación del receptor.

XX) Una proteína o polipéptido que comprende o consiste esencialmente, de dos o más dominios variables de inmunoglobulina (o fragmentos adecuados de los mismos) que son cada uno dirigidos contra epítopes en TNF-alfa (y en particular, del trímero TNF-alfa) que cae y/o forma parte del(los) sitio(s) de enlace al receptor del trímero de TNF, de manera tal que dicho polipéptido es capaz de enlazarse intramolecularmente con al menos, dos sitios de enlace del receptor de TNF en un trímero de TNF.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales, dichos dominios variables de inmunoglobulina están ligados entre sí, de forma tal que la proteína o

polipéptido es capaz de enlazarse simultáneamente a dos o más sitios de enlace del receptor en un trímero de TNF único.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales dichos dominios variables de inmunoglobulina son capaces de enlazarse al mismo epítope como Nanocuerpo TNF1 (SEC ID NO: 52).
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales dichos dominios variables de inmunoglobulina son capaces de enlazarse contra el epítope dentro del sitio de enlace del receptor de TNF del trímero de TNF que al menos, comprenden los siguientes residuos de aminoácidos: Gln en la posición 88 en el monómero A; Lys en la posición 90 en el monómero A y Glu en la posición 146 en el monómero B.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales dichos dominios variables de inmunoglobulina son capaces de enlazarse contra el epítope dentro del sitio de enlace del receptor de TNF del trímero de TNF que al menos, comprenden los siguientes residuos de aminoácidos: Gln en la posición 88 en el monómero A; Lys en la posición 90 en el monómero A y Glu en la

posición 146 en el monómero B; y que además comprende al menos uno, preferiblemente dos o más, más preferiblemente 5 ó más, y preferiblemente todos o esencialmente todos, de los siguientes residuos de aminoácidos del monómero A de TNF-alfa: Gly en la posición 24, Gln en la posición 25, Thr en la posición 72, His en la posición 73, Val en la posición 74, Leu en la posición 75, Thr en la posición 77, Thr en la posición 79, lie en la posición 83, Thr en la posición 89, Val en la posición 91. Asn en la posición 92, He en la posición 97, Arg en la posición 131, Glu en la posición 135, He en la posición 136, Asn en la posición 137, Arg en la posición 138, Pro en la posición 139, Asp en la posición 140 y los siguientes residuos en el monómero B: Pro en la posición 20, Arg en la posición 32, Lys en la posición 65, Lys en la posición 112, Tyr en la posición 115, Ala en la posición 145, Ser en la posición 147.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales dichos dominios variables de inmunoglobulina son capaces de enlazarse contra el epítope dentro del sitio de enlace del receptor de TNF del trímero de TNF que al menos, comprende los siguientes residuos de aminoácidos: Gln en la posición 88 en el monómero A;

Lys en la posición 90 en el monómero A y Glu en la posición 146 en el monómero B; y que además comprende al menos uno, preferiblemente dos o más, más preferiblemente 5 ó más, y preferiblemente todos o esencialmente todos, de los siguientes residuos de aminoácidos del monómero A de TNF-alfa Leu en la posición 75, Thr en la posición 77, Thr en la posición 79, He en la posición 80, Ser en la posición 81, Tyr en la posición 87, Thr en la posición 89, Val en la posición 91, Asn en la posición 92, Ser en la posición 95, He en la posición 97, Glu en la posición 135, He en la posición 136, Asn en la posición 137 y los siguientes residuos en el monómero B: Ala en la posición 33, Ala en la posición 145, Ser en la posición 147.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales al menos dos dominios variables de inmunoglobulina están ligados en tal forma que la distancia entre el N-término del primer dominio variable de inmunoglobulina y el C-término del segundo dominio variable de inmunoglobulina presente en tal proteína o polipéptido es al menos de 50 Angstroms.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales

la distancia entre el N-término del primer dominio variable de inmunoglobulina y el C-término del segundo dominio variable de inmunoglobulina es entre 55-200 Angstroms.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales la distancia entre el N-término del primer dominio variable de inmunoglobulina y el C-término del segundo dominio variable de inmunoglobulina es entre 65-150 Angstroms.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales el primero y segundo dominio variable de inmunoglobulina están ligados entre sí vía un enlazador o espaciador.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales el enlazador o secuencia es una secuencia de aminoácido.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales el enlazador o espaciador está comprendido de al menos 14 residuos de aminoácidos.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales

el enlazador o espaciador está comprendido de al menos 17 - 50 residuos de aminoácidos.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales el enlazador o espaciador consiste esencialmente de residuos de glicina y serina.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales el enlazador o espaciador es GS30 (SEC ID NO: 69).
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales el primero y segundo dominio variable de inmunoglobulina están ligados entre sí vía otra porción.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales dicha otra porción es una porción de proteína o polipéptido.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales dichas otras porciones confieren al menos, una propiedad deseada a la proteína o polipéptido, o mejora al menos, una propiedad deseada de la proteína o polipéptido.
- Una proteína o polipéptido de conformidad con

709

cualquiera de XIX) hasta XX) anteriores, en los cuales dicha otra porción mejora la vida media de la proteína o polipéptido y/o reduce la inmunogenicidad de la proteína o polipéptido.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales cada uno del primero y segundo dominio variable de inmunoglobulina están ligados a dicha otra porción vía un enlazador o espaciador.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales el enlazador o secuencia es una secuencia de aminoácido.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales el enlazador o espaciador consiste esencialmente de residuos de glicina y serina.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales dicha otra porción es un Nanocuerpo.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales la otra porción es un Nanocuerpo dirigido contra albúmina de suero humano.
- Una proteína o polipéptido de conformidad con

cualquiera de XIX) hasta XX) anteriores, en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humano es un Nanocuerpo humanizado.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humano es una variante humanizada del Nanocuerpo ALB 1 (SEC ID NO: 63).
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humano es uno elegido del grupo que consiste de ALB 3 (SEC ID NO: 87), ALB 4 (SEC ID NO: 88), ALB 5 (SEC ID NO: 89), ALB 6 (SEC ID NO: 100), ALB 7 (SEC ID NO: 101), ALB 8 (SEC ID NO: 102) ALB 9 (SEC ID NO: 103) y ALB 10 (SEC ID NO:104).
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humano es ALB 8.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos.
- Una proteína o polipéptido de conformidad con

cualquiera de XIX) hasta XX) anteriores, en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos con una relación Koff para TNF mejor de 2.10^{-3} (1/s), preferiblemente mejor que 1.10^{-3} (1/s); o una variante humanizada de tal Nanocuerpo.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos con un valor EC50 en el ensayo a base de células usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que el valor EC50 del Nanocuerpo VHH 3E (SEC ID NO:4) del documento WO 04/041862 en el mismo ensayo; o una variante humanizada de tal Nanocuerpo.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos con un valor EC50 en el ensayo a base de células usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que 12nM; o una variante humanizada de tal Nanocuerpo.
- Una proteína o polipéptido de conformidad con

cualquiera de XIX) hasta XX) anteriores, en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos con un valor EC50 en el ensayo a base de células usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que 5nM; o una variante humanizada de tal Nanocuerpo.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos con un valor EC50 en el ensáyo a base de células usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que 3nM; o una variante humanizada de tal Nanocuerpo.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos de conformidad con cualquiera de XIX) hasta XX) anteriores, los cuales son humanizados;

con algunos aspectos particularmente preferidos de esta modalidad siendo:

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales

713

el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos clase GLEW.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos con un residuo de arginina (R) en la posición 103.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos clase GLEW con un residuo de arginina (R) en la posición 103.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos con al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en este documento) con una de las secuencias de aminoácido de las SEC ID Nos. 52 (TNF1), 76 (TNF 13), 77 (TNF 14), 95 (TNF29) ó 96 (TNF30).
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales el primero y segundo dominios variables de

714

inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX) en los cuales:

a) CDR1 comprende:

- la secuencia de aminoácido DYWMY; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido DYWMY; o una secuencia de aminoácidos que tiene 2 o solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido DYWMY;

y

b) CDR2 comprende:

- la secuencia de aminoácido EINTNGLITKYPDSVKG; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido EINTNGLITKYPDSVKG; o
- una secuencia de aminoácidos que tiene 2 o solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido EINTNGLITKYPDSVKG;

y

c) CDR3 comprende:

715

- la secuencia de aminoácido SPSGFN; o

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido SPSGFN; o - una secuencia de aminoácidos que tiene 2 o solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido SPSGFN.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX) anteriores, en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX) en los cuales CDR1 comprende la secuencia de aminoácido DYWMY.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX) en los cuales CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son

Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX) en los cuales CDR3 comprende la secuencia de aminoácido SPSGFN.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX) en los cuales:
  - CDR1 comprende la secuencia de aminoácido DYWMY; y CDR3 comprende la secuencia de aminoácido SPSGFN; o
  - CDR1 comprende la secuencia de aminoácido DYWMY; y CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG; o
  - CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG; y CDR3 comprende la secuencia de aminoácido SPSGFN.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX) en los cuales CDR1 comprende la secuencia de aminoácido DYWMY; y CDR3 comprende la secuencia de

aminoácido SPSGFN.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX) en los cuales CDR1 comprende la secuencia de aminoácido DYWMY; CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG y CDR3 comprende la secuencia de aminoácido SPSGFN.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX) en los cuales:

a) CDR1 es:

- la secuencia de aminoácido DYWMY; o

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido DYWMY; o

- una secuencia de aminoácidos que tiene 2 o solamente 1 diferencia de aminoácido con la secuencia de

718

aminoácido DYWMY;

y en los cuales:

b) CDR2 es:

- la secuencia de aminoácido EINTNGLITKYPDSVKG; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido EINTNGLITKYPDSVKG; o
- una secuencia de aminoácidos que tiene 2 o solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido EINTNGLITKYPDSVKG;

y en los cuales

c) CDR3 es:

- la secuencia de aminoácido SPSGFN; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido SPSGFN; o
- una secuencia de aminoácidos que tiene 2 o solamente 1 diferencia de aminoácido con la secuencia de aminoácido SPSGFN.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero

719

y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX) en los cuales CDR1 es la secuencia de aminoácido DYWMY.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX) en los cuales CDR2 es la secuencia de aminoácido EINTNGLITKYPDSVKG.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describen para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX) en los cuales CDR3 es la secuencia de aminoácido SPSGFN.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX) en los cuales:

720

- CDR1 es la secuencia de aminoácido DYWMY; y CDR3 es la secuencia de aminoácido SPSGFN; o
- CDR1 es la secuencia de aminoácido DYWMY; y CDR2 es la secuencia de aminoácido EINTNGLITKYPDSVKG; o
- CDR2 es la secuencia de aminoácido EINTNGLITKYPDSVKG; y CDR3 es la secuencia de aminoácido SPSGFN.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX) en los cuales CDR1 es la secuencia de aminoácido DYWMY; y CDR3 es la secuencia de aminoácido SPSGFN.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX) en los cuales CDR1 es la secuencia de aminoácido DYWMY; CDR2 es la secuencia de aminoácido EINTNGLITKYPDSVKG y CDR3 es la secuencia de aminoácido SPSGFN.
- Una proteína o polipéptido de conformidad con

cualquiera de XDC) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX) en los cuales - cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativa; y/o dicha secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácidos, y no supresiones o inserciones de aminoácidos, comparada con la(s) secuencia(s) de aminoácido(s) anteriores.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son elegidos del grupo que consiste del Nanocuerpo TNF 1 (SEC ID NO: 52) y variantes humanizadas del Nanocuerpo TNF 1 (SEC ID NO: 52).
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son elegidos del grupo que consiste de TNF 13 (SEC ID NO: 76), TNF 14 (SEC ID NO: 77), TNF 29 (SEC ID NO: 95) y TNF 30 (SEC ID NO:96).
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero

y segundo dominios variables de inmunoglobulina son TNF 30 (SEC ID NO:96);

y con algunos aspectos particularmente preferidos de esta modalidad siendo:

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos clase KERE.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos con al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en este documento) con una de las secuencias de aminoácido de las SEC ID Nos. 50 (TNF3), 83 (TNF20), 85 (TNF21), 85 (TNF22), 96 (TNF23) ó 98 (TNF33).
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX) en los cuales:

a) CDR1 comprende:

723

- la secuencia de aminoácido NYYMG; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido NYYMG; o
- una secuencia de aminoácidos que tiene 2 o solamente 1 diferencia de aminoácido con la secuencia de aminoácido NYYMG;

y

b) CDR2 comprende:

- la secuencia de aminoácido NISWRGYNIYYKDSVKG; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido NISWRGYNIYYKDSVKG; o
- una secuencia de aminoácidos que tiene 2 o solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido NISWRGYNIYYKDSVKG;

y

c) CDR3 comprende:

- la secuencia de aminoácido SILPLSDDPGWNTY; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al

724

menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido SILPLSDDPGWNTY; o

- una secuencia de aminoácidos que tiene 2 o solamente 1 diferencia de aminoácido con la secuencia de aminoácido SILPLSDDPGWNTY.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX) en los cuales CDR1 comprende la secuencia de aminoácido NYYMG.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX) en los cuales CDR2 comprende la secuencia de aminoácido NISWRGYNIYYKDSVKG.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX)

hasta XX) en los cuales CDR3 comprende la secuencia de aminoácido SILPLSDDPGWNTY.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX), en los cuales:

  - CDR1 comprende la secuencia de aminoácido NYYMG; y CDR3 comprende la secuencia de aminoácido SILPLSDDPGWNTY; o

  - CDR1 comprende la secuencia de aminoácido NYYMG; y CDR2 comprende la secuencia de aminoácido NISWRGYNIYYKDSVKG; o

  - CDR2 comprende la secuencia de aminoácido NISWRGYNIYYKDSVKG; y CDR3 comprende la secuencia de aminoácido SILPLSDDPGWNTY.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX), en los cuales CDR1 comprende la secuencia de aminoácido NYYMG; CDR2 comprende la secuencia de aminoácido SILPLSDDPGWNTY y CDR3 comprende la

726

secuencia de aminoácido ILPLSDDPGWNTY (SEC ID NO:436).

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX), en los cuales

a) CDR1 es:

- la secuencia de aminoácido NYYMG; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido NYYMG; o
- una secuencia de aminoácidos que tiene 2 o solamente 1 diferencia de aminoácido con la secuencia de aminoácido NYYMG;

y

b) CDR2 es:

- la secuencia de aminoácido NISWRGYNIYYKDSVKG; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido NISWRGYNIYYKDSVKG; o

727

- una secuencia de aminoácidos que tiene 2 o solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido NISWRGYNIYYKDSVKG;

y

c) CDR3 es:

- la secuencia de aminoácido SILPLSDDPGWNTY; o

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido SILPLSDDPGWNTY; o

- una secuencia de aminoácidos que tiene 2 o solamente 1 diferencia de aminoácido con la secuencia de aminoácido SILPLSDDPGWNTY.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX), en los cuales CDR1 es la secuencia de aminoácido NYYMG.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o

728

polipéptidos de conformidad con cualquiera de XIX) hasta XX), en los cuales CDR2 es la secuencia de aminoácido NISWRGYNIYYKDSVKG.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX), en los cuales CDR3 es la secuencia de aminoácido SILPLSDDPGWNTY.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX), en los cuales:

  - CDR1 es la secuencia de aminoácido NYYMG; y CDR3 es la secuencia de aminoácido SILPLSDDPGWNTY; o

  - CDR1 es la secuencia de aminoácido NYYMG; y CDR2 es la secuencia de aminoácido NISWRGYNIYYKDSVKG; o

  - CDR2 es la secuencia de aminoácido NISWRGYNIYYKDSVKG; y CDR3 es la secuencia de aminoácido SILPLSDDPGWNTY.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero

729

y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX), en los cuales CDR1 es la secuencia de aminoácido NYYMG; CDR2 es la secuencia de aminoácido SILPLSDDPGWNTY y CDR3 es la secuencia de aminoácido ILPLSDDPGWNTY.

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son Nanocuerpos como se describe para las proteínas o polipéptidos de conformidad con cualquiera de XIX) hasta XX), en los cuales
  - cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativa; y/o
  - dicha secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácidos, y no supresiones o inserciones de aminoácidos, comparada con la(s) secuencia(s) de aminoácido(s) anteriores.
- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son elegidos del grupo que consiste del Nanocuerpo TNF 3 (SEC ID NO: 60) y variantes humanizadas del Nanocuerpo

TNF 3 (SEC ID NO: 60).

- Una proteína o polipéptido de conformidad con cualquiera de XIX) hasta XX), en los cuales el primero y segundo dominios variables de inmunoglobulina son elegidos del grupo que consiste de 83 TNF20 (SEC ID NO: 83), TNF21 (SEC ID NO: 84), TNF 22 (SEC ID NO: 85), TNF23 (SEC ID NO: 86) ó TNF33 (SEC ID NO: 99).

Se debe notar que, cuando una proteína o polipéptido es mencionado anteriormente por estar *"de conformidad con cualquiera de XIX) hasta XX) anteriores"*, está al menos, de conformidad con uno de XIX) a XX), y puede estar de conformidad con tanto XIX) como XX), y puede también incluir cualquiera o más de los otros aspectos que están indicados por estar "de conformidad con cualquiera de XIX) hasta XX) anteriores".

Sin embargo, se debe notar que la invención no está limitada a algún mecanismo de acción o hipótesis específica. En particular, se ha encontrado que los Nanocuerpos monovalentes de la invención, también pueden ser activos en los ensayos y modelos descritos en este documento, los cuales confirman que el enlace intramolecular del trímero de TNF, aunque se prefiere en una modalidad específica de la invención, no se requiere para obtener la acción y efecto deseado de los Nanocuerpos, proteínas y polipéptidos descritos en este documento. De

manera similar, también se abarcan dentro del alcance de la invención, que las proteínas y polipéptidos descritos aquí, logran su acción deseada vía cualquier mecanismo apropiado (por ejemplo, por enlace intramolecular, enlace intermolecular o aún, por enlace a TNF monomérico, de este modo, inhibiendo la formación de trímero de TNF).

También está dentro del campo de la invención, usar partes, fragmentos, análogos, mutantes, variantes, alelos y/o derivados de los Nanocuerpos y polipéptidos de la invención, y/o usar proteínas o polipéptidos que comprenden o consisten esencialmente de los mismos, tan pronto como estos sean adecuados para usos contemplados en este documento. Tales partes, fragmentos, análogos, mutantes, variantes, alelos, derivados, proteínas y/o polipéptidos, serán descritos en la descripción adicional en este documento.

En otro aspecto, la invención se refiere a un Nanocuerpo (como se define en este documento), contra TNF-alfa, el cual consiste de 4 regiones de estructura (FR1 a FR4, respectivamente) y 3 regiones que determinan la complementariedad (CDR1 a CDR3, respectivamente), en las cuales:

(i) CDR1 es una secuencia de aminoácido elegida del grupo que consiste de las secuencias CDR1 de las SEC ID NOS: 15 a 21 o del grupo que consiste de las secuencias

CDR1 de las SEC ID NOS: 164 a 197;

o del grupo que consiste de la secuencia de aminoácidos que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en este documento) con al menos una de las secuencias CDR1 del grupo que consiste de las SEC ID NOS: 15 a 21 ó con al menos una de las secuencias CDR1 del grupo que consiste de las SEC ID NOS: 164 hasta 197, en los cuales

(1) cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativa (como se define en este documento); y/o

(2) dicha secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácidos, y no supresiones o inserciones de aminoácidos, comparada con la(s) secuencia(s) de aminoácido(s) anteriores;

y/o del grupo que consiste de las secuencias de aminoácidos que tiene 2 ó solamente 1 "diferencia(s) de aminoácido(s)" (como se define en este documento) con al menos una de las secuencias CDR1 del grupo que consiste de las SEC ID NOS: 15 hasta 21 ó con al menos una de las secuencias CDR1 del grupo que consiste de las SEC ID NOS: 164 hasta 197, en las cuales:

(1) cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido

conservativa (como se define en este documento); y/o

(2) dicha secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácidos, y no supresiones o inserciones de aminoácidos, comparada con la(s) secuencia(s) de aminoácido(s) anteriores; y en las cuales:

(ii) CDR2 es una secuencia de aminoácido elegida del grupo que consiste de las secuencias CDR2 de las SEC ID NOS: 22 hasta 28 o del grupo que consiste de las secuencias CDR2 de las SEC ID NOS: 232 hasta 265;

o del grupo que consiste de las secuencias de aminoácidos que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en este documento) con al menos una de las secuencias CDR2 del grupo que consiste de las SEC ID NOS: 22 hasta 28 ó con al menos una de las secuencias CDR2 del grupo que consiste de las SEC ID NOS: 232 hasta 265, en las cuales

(1) cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativa (como se define en este documento); y/o

(2) dicha secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácidos, y no supresiones o inserciones de aminoácidos, comparada

con la(s) secuencia(s) de aminoácido(s) anteriores; y/o del grupo que consiste de las secuencias de aminoácidos que tienen 3, 2 ó solamente 1 "diferencia(s) de aminoácidos" (como se define en este documento) con al menos una de las secuencias CDR2 del grupo que consiste de las SEC ID NOS: 22 hasta 28 ó con al menos una de las secuencias CDR2 del grupo que consiste de las SEC ID NOS: 232 hasta 265, en las cuales:

(1) cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativa (como se define en este documento); y/o

(2) dicha secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácidos, y no supresiones o inserciones de aminoácidos, comparada con la(s) secuencia(s) de aminoácido(s) anteriores;

y en las cuales:

(iii) CDR3 es una secuencia de aminoácido elegida del grupo que consiste de las secuencias CDR3 de las SEC ID NOS: 29 hasta 33 o del grupo que consiste de las secuencias CDR3 de las SEC ID NOS: 300-333;

o del grupo que consiste de las secuencias de aminoácidos que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de

secuencia (como se define en este documento) con al menos una de las secuencias CDR3 del grupo que consiste de las SEC ID NOS: 29 hasta 33 ó con al menos una de las secuencias CDR3 del grupo que consiste de las SEC ID NOS: 300-333, en las cuales:

(1) cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativa (como se define en este documento); y/o

(2) dicha secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácidos, y no supresiones o inserciones de aminoácidos, comparada con la(s) secuencia(s) de aminoácido(s) anteriores; y/o

del grupo que consiste de las secuencias de aminoácidos que tienen 3, 2 ó solamente 1 "diferencia(s) de aminoácidos" (como se define en este documento) o con al menos una de las secuencias CDR3 del grupo que consiste de las SEC ID NOS: 300-333, en las cuales:

(1) cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativa (como se define en este documento); y/o

(2) dicha secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácidos, y no supresiones o inserciones de aminoácidos, comparada con la(s) secuencia(s) de aminoácido(s) anteriores;

o del grupo que consiste de las secuencias CDR3 de las SEC ID NOS: 34 y 35 ó del grupo que consiste de las secuencias de aminoácidos que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en este documento) con al menos una de las secuencias CDR3 de SEC ID NOS: 34 y 35, en las cuales:

(1) cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativa (como se define en este documento); y/o

(2) dicha secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácidos, y no supresiones o inserciones de aminoácidos, comparada con la(s) secuencia(s) de aminoácido(s) anteriores;

y/o del grupo que consiste de las secuencias de aminoácidos que tienen 3, 2 ó solamente 1 "diferencia(s) de aminoácidos" (como se define en este documento) con al menos una de las secuencias CDR3 de SEC ID NOS: 34 y 35, en las cuales:

(1) cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativa (como se define en este documento); y/o

(2) dicha secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácidos, y no

supresiones o inserciones de aminoácidos, comparada con la(s) secuencia(s) de aminoácido(s) anteriores.

Los Nanocuerpos contra TNF-alfa como se describe anteriormente, y como se describe además aquí posteriormente, también son referidos en este documento como Nanocuerpos de la invención.

De los Nanocuerpos de la invención, los Nanocuerpos que comprenden una o más de las CDR explícitamente listadas anteriormente, son particularmente preferidas; los Nanocuerpos que comprenden dos o más de las CDR explícitamente listadas anteriormente, son más particularmente preferidas; y los Nanocuerpos que comprenden tres de las CDR explícitamente listadas anteriormente, son más particularmente preferidas.

Algunas combinaciones particularmente preferidas, pero no limitantes de las secuencias de CDR, pueden verse en la Tabla I abajo, la cual lista las CDR y secuencias de estructuras que están presentes en un número de Nanocuerpos preferidos (pero no limitantes) de la invención. Como será claro para la persona experta, una combinación de secuencias CDR1, CDR2 y CDR3 que ocurren en el mismo clon (es decir, secuencias CDR1, CDR2 y CDR3, las cuales son mencionadas en la misma línea en la Tabla I), usualmente serán preferidas (aunque la invención en su sentido más amplio, no está limitada a esto, y también comprende otras

combinaciones adecuadas de las secuencias CDR mencionadas en la Tabla I).

También, en los Nanocuerpos de la invención que comprenden las combinaciones de CDR mencionadas en la Tabla I, cada CDR puede ser reemplazada por una CDR elegida del grupo que consiste de las secuencias de aminoácidos que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en este documento) con las CDR mencionadas; en las cuales

(1) cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativa (como se define en este documento); y/o

(2) dicha secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácidos, y no supresiones o inserciones de aminoácidos, comparada con la(s) secuencia(s) de aminoácido(s) anteriores;

y/o elegida del grupo que consiste de las secuencias de aminoácidos que tienen 3, 2 ó solamente 1 "diferencia(s) de aminoácido(s)" (como se indica en el párrafo precedente) (como se define en este documento) con una de las CDR(s) mencionadas de la secuencia de aminoácidos anterior, en la cual:

(1) cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido

conservativa (como se define en este documento); y/o

(2) dicha secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácidos, y no supresiones o inserciones de aminoácidos, comparada con la(s) secuencia(s) de aminoácido(s) anteriores.

Sin embargo, como será claro para las personas expertas, la (combinación de) secuencias de CDR mencionadas en la Tabla I, en general, será preferida.

Tabla 1: Combinaciones preferidas de estructura y secuencias de CDR

Clon	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
PMP1C2 (TNF1)	13 10	QVQLVESGGGLVQPG GSLRLSCAASGFTFS	16 14	DYWMY	19 18	WVRQAPGKG LEWVS	23 22	EINTNGLITKYP DSVKG	26 26	RFTISRDNAKNTLYLQMNSL KPEDTALYYCAR	30 30	SPSGFN	33 34	RGQGT QVTVSS
PMP1G11	13 11	QVQLQESGGGMVQPG GSLRLSCAASGFDFG	16 15	VSWMY	19 19	WVRQAPGKG LEWVS	23 23	EINTNGLITKYP DSVKG	26 27	RFTISRDNAKTTLYLQMNSL KPEDTALYYCAR	30 31	SPSGSF	33 35	RGQGT QVTVSS
PMP1H6	13 12	EVQLVESGGGLVQPG GSLRLSCATSGFDFS	16 16	VSWMY	20 20	WVRQAPGKG LEWVS	23 24	EINTNGLITKYV DSVKG	26 28	RFTISRDNAKNTLYLQMDSL IPEDTALYYCAR	30 32	SPSGSF	33 36	RGQGT QVTVSS
PMP1G5 (TNF2)	13 13	QVQLVESGGGLVQAG GSLRLSCAASGRTFS	16 17	EPSGYT YTIG	20 21	WFRQAPGKE REFVA	23 25	RIYWSSGLTYY ADSVKG	26 29	RFTISRDIAKNTVDLLMNSL KPEDTAVYYCAA	30 33	RDGIPTSRSV GSYNY	33 37	WGQGT QVTVSS
PMP1H2	13 14	QVKLEESGGGLVQPG DSLRLSCAASGRTFS	16 18	DYSGYT YTVG	20 22	WFRQAPGKE REFVA	23 26	RIYWSSGNTYY ADSVKG	27 30	RFTISRDIAKNTVDLLMNNL EPEDTAVYYCAA	30 34	RDGIPTSRSV ESYNY	33 38	WGQGT QVTVSS
PMP3G2	13 15	AVQLVESGGGLVQPG DSLRLSCAASGRTFS	16 19	DYSGYT YTVG	20 23	WFRQAPGKE REFVA	23 27	RIYWSSGNTYY ADSVKG	27 31	RFTISRDIAKNTVDLLMNNL EPEDTAVYYCAA	30 35	RDGIPTSRSV ESYNY	33 39	WGQGT QVTVSS
PMP1D2	13 16	AVQLVDSGGGLVQAG GSLRLSCAASGRTFS	17 20	AHSVYT MG	20 24	WFRQAPGKE REFVA	23 28	RIYWSSANTYY ADSVKG	27 32	RFTISRDNAKNTVDLLMNCL KPEDTAVYYCAA	30 36	RDGIPTSRSV EAYNY	34 40	WGQGT QVTVSS

Tabla I (continuación):

Clon	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
PMP3D10	13 7	QVQLVESGGGLVQAGG SLSLSCAASGRSFT	17 1	GYYMG	20 5	WFRQAPG KERQLLA	23 9	SISWRGDNTYYKE SVKG	27 3	RFTISRDDAKNTIYLQMN SLKPEDTAVYYCAA	30 7	SILPLSDDPG WNTN	34 1	WGQG TQVTV SS
PMP5F10 (TNF3)	13 8	EVQLVESGGGLVQAGG SLSLSCSASGRSLS	17 2	NYYMG	20 6	WFRQAPG KERELLG	24 0	NISWRGYNIYYKDS VKG	27 4	RFTISRDDAKNTIYLQMN RLKPEDTAVYYCAA	30 8	SILPLSDDPG WNTY	34 2	WGQG TQVTV SS
NC55TNF_ S1C4	13 9	EVQLVESGGGLVQAGD SLRLSCAASQIIFG	17 3	SHVAA	20 7	WFRQAPG REREFVA	24 1	EIRPSGDFGPEGE FEHVTASLKG	27 5	RFTIAKNSVDNTVYLQM NSLKPEDTAVYYCAA	30 9	APYRGGRDYR WEYEYEY	34 3	WGQG TQVTV SS
NC55TNF_ S1C3	14 0	EVQLVESGGGLVQPGG SLRLSCKNAGSTSN	17 4	AYATG	20 8	WFRRAPG KEREFVA	24 2	GIQWSGGDAFYRN SVKG	27 6	RFRITRDPDNTVYLQMN DLKPEDTAIYYCAQ	31 0	KLSPYYNDFD SSNYEY	34 4	WGQG TQVTV SS
NC55TNF_ S2C1	14 1	EVQLVESGGDLVQPGG SLRLSCAVSGQLFS	17 5	TNDVG	20 9	WYRRAPG KQRELVA	24 3	TITDDGTTDYGDD VKG	27 7	RFVISREGEMVYLEMNS LKPEDTAVYYCNI	31 1	NRLRSTWGIR YDV	34 5	WGQG TQVTV SS
NC55TNF_ S2C5	14 2	EVQLVESGGGLVQPGG SLRLSCVVSGFTFS	17 6	TTSMT	21 0	WVRQAPG KFEEWVS	24 4	FINSDGSSTTYADS VKG	27 8	RFTISRDNAKNTLYLQMN SLKPEDTAMYYCGR	31 2	RGYGRD	34 6	RSKGI QVTVA S
NC55TNF_ S3C7	14 3	EVQLVESGGGTVQAGD SLRLSCAASGRSFS	17 7	SVAMG	21 1	WFRQAPG KQREFLA	24 5	GVGYDGSSIRYAE SVKG	27 9	RFTIARGNRESTVFLQM ENLKPEDTAVYFCTA	31 3	EPIGAYEGLW TY	34 7	WGQG TQVTV SS

Tabla I (continuación):

Clon	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
NC55TNF_S3C1	14	TNF-ALPHAVESGGGLMQPGGSLKLSCAASGFMFS	178	DSAMG	21	WFRQAPGKEREFVA	246	TISWNGGSSSYADFVKG	288	RFTISRDNAKNTVYLQMNGLTPQDTAIYYCAG	314	SYSNGNPHRFSQYQY	348	WGQGTQVTVSS
NC55TNF_BMP1B2	145	EVQLVESGGGLVQAGGSLRLSCAASGRTFG	179	TYAMG	31	WFRQAPGKEREFVA	247	AISWGGGSIVYAESAKG	281	RFTISRDNAKXTMYLQMDSLKPEDTAVYYCAA	315	ANNIATLRQGS	349	WGQGTQVTVSS
NC55TNF_BMP1D2	146	EVQLVESGGELVQAGGSLKLSCTASGRNFV	180	TYAMS	41	WFRRAPGKEREFVA	248	SISWSGDTTYYSNSVKG	282	RFTVSRDNGKNTAYLRMNSLKPEDTADYYCAV	316	VQVIDPSWSGVNLDDYDY	350	LGSGTQVTVSS
NC55TNF_BMP1E2	147	EVQLVESGGRLVQPGGSLRLSCKNAGSTSN	181	AYATG	51	WFRRAPGKEREFVA	249	GIQWSGGDAFYRNSVKG	283	RFRITRDPDNTVYLQMNDLKPEDTAIYYCAQ	317	KLSPYYNDFDSSNYEY	351	WGQGTQVTVSS
NC55TNF_BMP1G2	148	EVQLVESGGGLVQPGGSLRLSCAASATISS	182	IVMLG	61	WYRQAPGKQREWVA	250	SITIGSRTNYADSVKG	284	RFTISRDNAKNTVYLQMNSLKPEDTAVYFCNA	318	VPPRDDY	352	WGQGTQVTVSS
NC55TNF_BMP2A2	149	EVQLVESGGGLVQAGGSLRLSCAASGQTSS	183	SYDMG	71	WFRQAPGEGREFVA	251	RISGSDGSTYYSDRAKD	285	RFTISRDNTKNMVYLQMDRLKPDDTAVYYCRV	319	PRYENQWSSYDY	353	WGQGTQVTVSS
NC55TNF_BMP2C2	150	EVQLVESGGGLVQPGGSLRLSCAASGSTFS	184	TYDMS	81	WVRQAPGKGLEWVS	252	GIDSGGGSPMYVDSVKG	286	RFTVSRDNAKNTLYLQMNSLKPEDTAVYYCAK	320	FSTGADGGSWYWSYGMDS	354	WGKGTQVTVSS

Tabla I (continuación):

Clon	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
NC55TNF_BMP2F2	15	EVQLVESGGGLVQAG	85	RYNMA	21	WFRQAPGK	53	RVDVSGGNT	87	RFTVSRINGKNAMYLQMNNL	25	GGWGTTQYDY	35	WGQG TQVTV SS
NC55TNF_NC10	15	EVQLVESGGGLVQPG	82	FSAYSMT	22	WVRQAPGK	54	FINSDGSSTT	87	RFKISRDNAKKTLYLQMNSL	25	RGYALD	35	RGQGT QVTVS S
NC55TNF_NC11	15	EVQLVESGGGLVQAG	87	KNAMG	22	WFRQPPGK	53	SIKWNGNNT	87	RFTISRGNAKNTENTVSLQM	25	DSSHYSYVYSK	35	WGQG TQVTV SS
NC55TNF_NC1	15	EVQLVESGGGLVQPG	82	ASSMA	22	WVRQAPGK	53	FINSDGSSTT	87	RFTISRDNAKNTLYLQMNSL	25	AYEYDY	37	RSQGI QVTVS S
NC55TNF_NC2	15	EVQLVESGGGLVQAG	89	SYAMG	23	WFRQAPGK	57	AISWSGTITN	91	RFTISRDNGKNTVHLQMNSL	25	VQPYSGGDYY	35	WGXG TQVTV SS
NC55TNF_NC3	15	EVQLVESGGGLVQPG	90	ATSMT	24	WVRQAPGK	58	FINSDGSSTT	92	RFTISRDNAKNTLYLQMDDL	26	RGYGRD	60	RSRGI QVTVS S
NC55TNF_NC5	15	EVQLVESGGGLVQAG	91	NYDVG	25	WFRQAPGE	59	RISGSGDST	93	RFTISRDNAKNTVYLQMNSL	27	ARYNGTWSSN DY	61	WGQG TQVTV SS

Tabla I (continuación):

Clon	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
NC55TN F_NC6	15 8	EVQLVESGGGLVQPGGSL RLSCECVSSGCT	19 2	FSAYSM T	22 6	WVRQAPGKA EEFVS	26 0	FINSDGSSTT YANSVNG	29 4	RFKISRDNAKKTLYLQMNSL GPEDTAMYYCQR	32 8	RGYALD	36 2	RGQGTQ VTVSS
NC55TN F_NC7	15 9	QVQLVESGGGLVQAGGS LRLSCTASGQTSS	19 3	TADMG	22 7	WFRQPPGKG REFVA	26 1	RISGIDGTTY YDEPVKG	29 5	RFTISRDKAQNTVYLQMDSL KPEDTAVYYCRS	32 9	PRYADQW SAYDY	36 3	WGQGTQ VTVSS
NC55TN F_NC8	16 0	EVQLVESGGGLVQPGGSL RLSCVVSGFTFS	19 4	TTSMT	22 8	WVRQAPGKF EEWVS	26 2	FINSDGSSTT YADSVKG	29 6	RFTISRDNAKNTLYLQMNSL KPEDTAMYYCGR	33 0	RGYGRD	36 4	RSKGIQV TVSS
NC55TN F_S2C2	16 1	EVQLVESGGGLVQPGGSL RLSCVASASGVK	19 5	VNDMG	22 9	WYRQAPGKE RELVA	26 3	TITDDGRTNY EDFAKG	29 7	RFTISRDNAKNTVYLQMNSL LPEDTAVYYCNA	33 1	RTYWAHL PTY	36 5	WGQGTQ VTVSS
NC55TN F_S1C6	16 2	EVQLVESGGGLVQAGGSL RLSCAASGRSFG	19 6	SVAMG	23 0	WFRQAPGKE REFVA	26 4	AIGYDGNSIR YGDSVKG	29 8	RFTISRDNIKNTMYLEMENL NADDTARYLCAA	33 2	EPLARYE GLWTY	36 6	WGQGTQ VTVSS
NC55TN F_S3C2	16 3	EVQLVESGGGLVQAGASL RLSCTTSTRTN	19 7	DRFNMA	23 1	WFHQAPGKD REFVS	26 5	RIDVAGYNTA YGDFVKG	29 9	RFTVSRDSAENTVVLQMNSL RPEDTGVYYCAA	33 3	GGWGISQ SDYDL	36 7	WGQGTQ VTVSS

Notas a la Tabla I:

- ID se refiere a las SEC ID NOs. En el listado de secuencia adjunto.

- Para CDR1: La SEC ID NO:164 corresponde a la SEC ID NO:15, la SEC ID NO:167 corresponde a la SEC ID NO:16; la SEC ID NO:172 corresponde a la SEC ID NO:17, las SEC ID NOS: 165 y 166, corresponden a la SEC ID NO:18, la SEC ID NO:170, corresponde a la SEC ID NO:19, la SEC ID NO:171 corresponde a la SEC ID NO:20, y las SEC ID NOS: 168 y 169, corresponden a la SEC ID NO:21.

- Para CDR2: Las SEC ID NOS:232 y 233, corresponden a la SEC ID NO:22, la SEC ID NO:235 corresponde a la SEC ID NO:23, la SEC ID NO:240 corresponde a la SEC ID NO:24, la SEC ID NO:234 corresponde a la SEC ID NO:25, las SEC ID NOS:236 y 237, corresponden a la SEC ID NO:6, la SEC ID NO:238 corresponde a la SEC ID NO:27, y la SEC ID NO:239 corresponde a la SEC ID NO:28.

- Para CDR3: La SEC ID NO:303 corresponde a la SEC ID NO:29, la SEC ID NO:308 corresponde a la SEC ID NO:30, la SEC ID NO:306 corresponde a la SEC ID NO:31, la SEC ID NO:307 corresponde a la SEC ID NO:32, las SEC ID NOS: 304 y 305 corresponden a la SEC ID NO:33, la SEC ID NO:300 corresponde a la SEC ID NO:34, y las SEC ID NO: 301 y 302 corresponden a la SEC ID NO:35.

De este modo, en los Nanocuerpos de la invención, al menos una de las secuencias CDR1, CDR2 y CDR3 presentes, es adecuadamente elegida del grupo que consiste de las Secuencias CDR1, CDR2 y CDR3, respectivamente, listadas en la Tabla I; o del grupo de las secuencias CDR1, CDR2 y CDR3, respectivamente, que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de "identidad de secuencia" (como se define en este documento) con al menos una de las secuencias CDR1, CDR2 y CDR3, respectivamente, listadas en la Tabla I; y/o del grupo que consiste de las secuencias CDR1, CDR2 y CDR3, respectivamente, que tienen 3, 2 ó solamente 1 "diferencia(s) de aminoácido(s)" (como se define en este documento) con al menos una de las secuencias CDR1, CDR2 y CDR3, respectivamente, listadas en la Tabla I. En este contexto, por "elegida adecuadamente", significa que, conforme es aplicable, una secuencia CDR1 es elegida de las secuencias CDR1 adecuadas (es decir, como se define en este documento), una secuencia CDR2 es elegida de las secuencias CDR2 adecuadas (es decir, como se define en este documento) y una secuencia CDR3 es elegida de las secuencias CDR3 adecuadas (es decir, como se define en este documento), respectivamente.

En particular, en los Nanocuerpos de la invención, al menos la secuencia CDR3 presente es

adecuadamente elegida del grupo que consiste de las secuencias CDR3 listadas en la Tabla I o del grupo de secuencias CDR3 que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con al menos una de las secuencias CDR3 listadas en la Tabla I; y/o del grupo que consiste de las secuencias CDR3 que tienen 3, 2 ó solamente 1 diferencia(s) de aminoácido(s) con al menos una de las secuencias CDR3 listadas en la Tabla I.

Preferiblemente, en los Nanocuerpos de la invención, al menos dos de las secuencias CDR1, CDR2 y CDR3 presentes son adecuadamente elegidas del grupo que consiste de las secuencias CDR1, CDR2 y CDR3, respectivamente, listadas en la Tabla I o del grupo que consiste de las secuencias CDR1, CDR2 y CDR3, respectivamente, que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con al menos una de las secuencias CDR1, CDR2 y CDR3, respectivamente, listadas en la Tabla I; y/o del grupo que consiste de las secuencias CDR1, CDR2 y CDR3, respectivamente, que tienen 3, 2 ó solamente 1 "diferencia(s) de aminoácido(s)" con al menos una de las secuencias CDR1, CDR2 y CDR3, respectivamente, listadas en la Tabla I.

En particular, en los Nanocuerpos de la invención, al menos la secuencia CDR3 presente es adecuadamente elegida del grupo que consiste de las secuencias CDR3 listadas en la Tabla I o del grupo de secuencias CDR3 que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con al menos una de las secuencias CDR3 listadas en la Tabla I, respectivamente; y al menos una de las secuencias CDR1 y CDR2 presentes es adecuadamente elegida del grupo que consiste de las Secuencias CDR1 y CDR2, respectivamente, listadas en la Tabla I o del grupo de secuencias CDR1 y CDR2, respectivamente, que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con al menos una de las secuencias CDR1 y CDR2, respectivamente, listadas en la Tabla I; y/o del grupo que consiste de las secuencias CDR1 y CDR2, respectivamente, que tienen 3, 2 ó solamente 1 diferencia(s) de aminoácido(s) con al menos una de las secuencias CDR1 y CDR2, respectivamente, listadas en la Tabla I. Más preferiblemente, en los Nanocuerpos de la invención, las tres secuencias CDR1, CDR2 y CDR3 presentes son adecuadamente elegidas del grupo que consiste de las secuencias CDR1, CDR2 y CDR3, respectivamente, listadas en

la Tabla I o del grupo de las secuencias CDR1, CDR2 y CDR3, respectivamente, que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con al menos una de las secuencias CDR1, CDR2 y CDR3, respectivamente, listadas en la Tabla I; y/o del grupo que consiste de las secuencias CDR1, CDR2 y CDR3, respectivamente, que tienen 3, 2 ó solamente 1 diferencia(s) de aminoácido(s) con al menos una de las secuencias CDR1, CDR2 y CDR3, respectivamente, listadas en la Tabla I.

Aún más preferiblemente, en los Nanocuerpos de la invención, al menos una de las secuencias CDR1, CDR2 y CDR3 presentes, es adecuadamente elegida del grupo que consiste de las Secuencias CDR1, CDR2 y CDR3, respectivamente, listadas en la Tabla I. Preferiblemente, en esta modalidad, al menos una ó preferiblemente ambas otras dos secuencias de CDR presentes son adecuadamente elegidas de las secuencias CDR que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con al menos una de las secuencias CDR correspondientes, respectivamente, listadas en la Tabla I; y/o del grupo que consiste de las secuencias CDR que tienen 3, 2 ó solamente 1 diferencia(s) de aminoácido(s) con al menos una de las

secuencias correspondientes, respectivamente, listadas en la Tabla I.

En particular, en los Nanocuerpos de la invención, al menos la secuencia CDR3 presente es adecuadamente elegida del grupo que consiste de las CDR3 listadas en la Tabla I. Preferiblemente, en esta modalidad, al menos una y preferiblemente ambas de las secuencias CDR1 y CDR2 presente son adecuadamente elegidas de los grupos de secuencias CDR1 y CDR2, respectivamente, que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con las secuencias CDR1 y CDR2, respectivamente, listadas en la Tabla I, y/o del grupo que consiste de las secuencias CDR1 y CDR2, respectivamente, que tienen 3, 2 ó solamente 1 diferencia(s) de aminoácido(s) con al menos una de las secuencias CDR1 y CDR2, respectivamente, listadas en la Tabla I.

Aún más preferiblemente, en los Nanocuerpos de la invención, al menos dos de las secuencias CDR1, CDR2 y CDR3 presentes son adecuadamente elegidas del grupo que consiste de las secuencias CDR1, CDR2 y CDR3, respectivamente, listadas en la Tabla I. Preferiblemente, en esta modalidad, la secuencia CDR restante presente es adecuadamente elegida del grupo de secuencias CDR que tienen al menos 80%,

preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con al menos una de las secuencias CDR correspondientes listadas en la Tabla I, y/o del grupo que consiste de secuencias CDR que tienen 3, 2 ó solamente 1 diferencia(s) de aminoácido(s) con al menos una de las secuencias correspondientes listadas en la Tabla I.

En particular, en los Nanocuerpos de la invención, al menos la secuencia CDR3 es adecuadamente elegida del grupo que consiste de las secuencias CDR3 listadas en la Tabla I, y ya sea la secuencia CDR1 ó la secuencia CDR2 es adecuadamente elegida del grupo que consiste de las secuencias CDR1 y CDR2, respectivamente, listadas en la Tabla I. Preferiblemente, en esta modalidad, la secuencia CDR restante presente es adecuadamente elegida del grupo de secuencias CDR que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con al menos una de las secuencias CDR correspondientes listadas en la Tabla I, y/o del grupo que consiste de secuencias CDR que tienen 3, 2 ó solamente 1 diferencia(s) de aminoácido(s) con las secuencias CDR correspondientes listadas en la Tabla I.

Aún más preferiblemente, en los Nanocuerpos de la invención, las tres secuencias CDR1, CDR2 y CDR3 presentes

son adecuadamente elegidas del grupo que consiste de las secuencias CDR1, CDR2 y CDR3, respectivamente, listadas en la Tabla I.

También, en general, las combinaciones de CDR listadas en la Tabla I (es decir, aquellas mencionadas en la misma línea en la Tabla I) son preferidas. De este modo, se prefiere en general que, cuando una CDR en un Nanocuerpo de la invención es una secuencia CDR mencionada en la Tabla I ó es adecuadamente elegida del grupo de secuencias CDR que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con una secuencia CDR listada en la Tabla I, y/o del grupo que consiste de secuencias CDR que tienen 3, 2 ó solamente 1 diferencia(s) de aminoácido(s) con una secuencia CDR listada en la Tabla I, que al menos una y preferiblemente ambas de las otras CDRs son adecuadamente elegidas de las secuencias CDR que pertenecen a la misma combinación en la Tabla I (es decir, mencionadas en la misma línea en la Tabla I) ó son adecuadamente elegidas del grupo de secuencias CDR que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con las secuencias CDR que pertenecen a la misma combinación y/o del grupo que consiste de secuencias CDR que tienen 3, 2 ó solamente 1

diferencia(s) de aminoácido(s) con la(s) secuencia(s) CDR que pertenecen a la misma combinación. Las otras preferencias indicadas en los párrafos anteriores también aplican a las combinaciones de CDR mencionadas en la Tabla I.

De este modo, por medio de ejemplos no limitantes, un Nanocuerpo de la invención puede por ejemplo, comprender una secuencias CDR1 que tiene más de 80% de identidad de secuencia con una de las secuencias CDR1 mencionadas en la Tabla I, una secuencia CDR2 que tiene 3, 2 ó 1 diferencia de aminoácido con una de las secuencias CDR2 mencionadas en la Tabla I (pero que pertenecen a una combinación diferente), y una secuencia CDR3.

Algunos Nanocuerpos preferidos de la invención pueden, por ejemplo, comprender: (1) una secuencia CDR1 que tiene más de 80 % de identidad de secuencia con una de las secuencias CDR1 mencionadas en la Tabla I; una secuencia CDR2 que tiene 3, 2 ó 1 diferencias de aminoácido con una de las secuencias CDR2 mencionadas en la Tabla I (pero que pertenecen a una combinación diferente); y una secuencia CDR3 que tiene más de 80 % de identidad de secuencia con una de las secuencias CDR3 mencionadas en la Tabla I (pero que pertenecen a una combinación diferente); ó (2) una secuencia CDR1 que tiene

más de 80 % de identidad de secuencia con una de las secuencias CDR1 mencionadas en la Tabla I; una secuencia CDR2, y una de las secuencias CDR3 listadas en la Tabla I; ó (3) una secuencia CDR1; una secuencia CDR2 que tiene más de 80% de identidad de secuencia con una de las secuencias CDR2 listadas en la Tabla I; y una secuencia CDR3 que tiene 3, 2 ó 1 diferencias de aminoácidos con la secuencia CDR3 mencionada en la Tabla I que pertenece a la misma combinación como la secuencia CDR2.

Algunos Nanocuerpos particularmente preferidos de la invención pueden por ejemplo, comprender: (1) una secuencia CDR1 que tiene más de 80 % de identidad de secuencia con una de las secuencias CDR1 mencionadas en la Tabla I; una secuencia CDR2 que tiene 3, 2 ó 1 diferencias de aminoácido con la secuencia CDR2 mencionada en la Tabla I que pertenece a la misma combinación; y una secuencia CDR3 que tiene más de 80 % de identidad de secuencia con la secuencia CDR3 mencionada en la Tabla I que pertenece a la misma combinación; (2) una secuencia CDR1; una CDR2 listada en la Tabla I y una secuencia CDR3 listada en la Tabla I (en las cuales la secuencia CDR2 y La secuencia CDR3 pueden pertenecer a combinaciones diferentes).

Algunos Nanocuerpos aún más preferidos de la invención pueden por ejemplo, comprender: (1) una secuencia CDR1 que tiene más de 80 % de identidad de secuencia con

una de las secuencias CDR1 mencionadas en la Tabla I; la secuencia CDR2 listada en la Tabla I que pertenece a la misma combinación; y una secuencia CDR3 mencionada en la Tabla I que pertenece a una combinación diferente; ó (2) una secuencia CDR1 mencionada en la Tabla I; una secuencia CDR2 que tiene 3, 2 ó 1 diferencias de aminoácidos con la secuencia CDR2 mencionada en la Tabla I que pertenece a la misma combinación; y más de 80% de identidad de secuencia con la secuencia CDR3 listada en la Tabla I que pertenece a la misma combinación.

Los Nanocuerpos particularmente preferidos de la invención pueden por ejemplo, comprender una secuencia CDR1 mencionada en la Tabla I, una secuencia CDR2 que tiene más de 80 % de identidad de secuencia con la secuencia CDR2 mencionada en la Tabla I que pertenece a la misma combinación; y la secuencia CDR3 mencionada en la Tabla I que pertenece a la misma.

En lo más preferido en los Nanocuerpos de la invención, las secuencias CDR1, CDR2 y CDR3 presentes son adecuadamente elegidas de una de las combinaciones de secuencias CDR1, CDR2 y CDR3, respectivamente, listadas en la Tabla I.

Preferiblemente, cuando una secuencia CDR es adecuadamente elegida del grupo de secuencias CDR que tienen al menos 80%, preferiblemente al menos 90%, más

preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en este documento) con una de las secuencias CDR listadas en la Tabla I; y/o cuando una secuencia CDR es adecuadamente elegida del grupo que consiste de secuencias CDR que tienen 3, 2 ó solamente 1 diferencia(s) de aminoácido(s) con una de las secuencias CDR listadas en la Tabla I:

i) cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativa (como se define en este documento); y/o

ii) dicha secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácidos, y no supresiones o inserciones de aminoácidos, comparadas con las secuencias CDR listadas en la Tabla I.

De conformidad con una modalidad no limitante pero preferida de la invención, las secuencias de CDR en los Nanocuerpos de la invención son como se definen anteriormente y también son tales que el Nanocuerpo de la invención se enlaza a TNF-alfa con una constante de disociación (K_D) de 10-5 hasta 10^{-12} moles/litro (M) o menos, y preferiblemente, 10^{-7} a 10^{-12} moles/litro (M) o menos y más preferiblemente, 10^{-8} hasta 10^{-12} moles/litro (M), y/o una constante de disociación (K_A) de al menos 10^7 M^{-1}, preferiblemente al menos 10^8 M^{-1}, más preferiblemente al menos 10^9 M^{-1}, tal como al menos 10^{12} M^{-1}; y en particular,

con una K_D menos de 500 nM, preferiblemente, menos de 200 nM, más preferiblemente, menos de 10 nM, tal como menos de 500 nM. Los valores K_D y K_A del Nanocuerpo de la invención contra TNF-alfa, pueden ser determinados en una manera conocida per se, por ejemplo, usando el ensayo descrito en este documento.

De conformidad con otra modalidad preferida, pero no limitante de la invención, (a) CDR1 tiene una longitud de entre 1 y 12 residuos de aminoácido, y usualmente entre 2 y 9 residuos de aminoácidos, tales como 5, 6 ó 7 residuos de aminoácidos; y/o (b) CDR2 tiene una longitud de entre 13 y 24 residuos de aminoácido, y usualmente entre 15 y 21 residuos de aminoácido, tal como 16 y 17 residuos de aminoácido; y/o (c) CDR3 tiene una longitud de entre 2 y 35 residuos de aminoácido, y usualmente entre 3 y 30 residuos de aminoácido, tales como entre 6 y 23 residuos de aminoácido.

En un aspecto, la invención proporciona Nanocuerpos contra TNF-alfa que son de mejor desempeño que el Nanocuerpo 3E, el mejor desempeño del Nanocuerpo de conformidad con el documento WO 04/041862.

Más específicamente, algunos aspectos preferidos de esta modalidad de la invención son:

XXI) Un Nanocuerpo contra TNF-alfa, el cual consiste de 4 regiones de estructura (FR1 hasta FR4

respectivamente) y 3 regiones que determinan la complementariedad (CDR1 hasta CDR3 respectivamente), la cual tiene una relación Koff de TNF mejor de 2.10-3 (1/s), preferiblemente mejor que 1.10-3 (1/s); o una variante humanizada de tal Nanocuerpo.

XXII) Un Nanocuerpo contra TNF-alfa, el cual consiste de 4 regiones de estructura (FR1 hasta FR4 respectivamente) y 3 regiones que determinan la complementariedad (CDR1 hasta CDR3 respectivamente), la cual tiene un valor EC50 en el ensayo a base de células usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que el valor EC50 del Nanocuerpo VHH 3E (SEC ID NO:4) del documento WO 04/041862 en el mismo ensayo; o una variante humanizada de tal Nanocuerpo.

XXIII) Un Nanocuerpo contra TNF-alfa, el cual tiene un valor EC50 en el ensayo a base de células usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que 12nM; o una variante humanizada de tal Nanocuerpo.

XXIV) Un Nanocuerpo contra TNF-alfa, el cual tiene un valor EC50 en el ensayo a base de células usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que 5nM; o una variante humanizada de tal Nanocuerpo.

XXV) Un Nanocuerpo contra TNF-alfa, el cual tiene un valor EC50 en el ensayo a base de células usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que 3nM; o una variante humanizada de tal Nanocuerpo; con algunos aspectos particularmente preferidos siendo:

- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), el cual es un Nanocuerpo clase GLEW.
- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), el cual contiene un residuo de arginina (R) en la posición 103.
- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), el cual es un Nanocuerpo humanizado.
- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), el cual contiene un residuo leucina (L) en la posición 108.
- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), el cual tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en este documento) con una de las secuencias de aminoácido de las SEC ID Nos. 52 (TNF1), 76 (TNF13), 77 (TNF14), 95 (TNF29) ó 96 (TNF30).
- Un Nanocuerpo de conformidad con cualquiera de XXI)

hasta XXV), en los cuales

a) CDR1 comprende:

- la secuencia de aminoácido DYWMY; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido DYWMY; o
- una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido DYWMY;

y

b) CDR2 comprende:

- la secuencia de aminoácido EINTNGLITKYPDSVKG; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido EINTNGLITKYPDSVKG; o una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido EINTNGLITKYPDSVKG;

y

c) CDR3 comprende:

- la secuencia de aminoácido SPSGFN; o
- una secuencia de aminoácido que tiene al menos 80%,

preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido SPSGFN; o

- una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido SPSGFN.

- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales CDR1 comprende la secuencia de aminoácido DYWMY.

- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG.

- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales CDR3 comprende la secuencia de aminoácido SPSGFN

- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales:

  - CDR1 comprende la secuencia de aminoácido DYWMY; y CDR3 comprende la secuencia de aminoácido SPSGFN; o

  - CDR1 comprende la secuencia de aminoácido DYWMY; y CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG; ó

  - CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG; y CDR3 comprende la secuencia de

aminoácido SPSGFN.

- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales CDR1 comprende la secuencia de aminoácido DYWMY; y CDR3 comprende la secuencia de aminoácido SPSGFN.
- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales CDR1 comprende la secuencia de aminoácido DYWMY; CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG y CDR3 comprende la secuencia de aminoácido SPSGFN.
- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales

a) CDR1 es:

- la secuencia de aminoácido DYWMY; ó
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido DYWMY; o
- una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia de aminoácido con la secuencia de aminoácido DYWMY;

y en las cuales:

b) CDR2 es:

- la secuencia de aminoácido EINTNGLITKYPDSVKG; o

762

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido EINTNGLITKYPDSVKG; o una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido EINTNGLITKYPDSVKG;

y en las cuales

c) CDR3 es:

- la secuencia de aminoácido SPSGFN; o

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido SPSGFN; o

- una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia de aminoácido con la secuencia de aminoácido SPSGFN.

- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales CDR1 es la secuencia de aminoácido DYWMY.

- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales CDR2 es la secuencia de aminoácido EINTNGLITKYPDSVKG.

- Un Nanocuerpo de conformidad con cualquiera de XXI)

hasta XXV), en los cuales CDR3 es la secuencia de aminoácido SPSGFN.

- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales:
  - CDR1 es la secuencia de aminoácido DYWMY; y CDR3 es la secuencia de aminoácido SPSGFN; o
  - CDR1 es la secuencia de aminoácido DYWMY; y CDR2 es la secuencia de aminoácido EINTNGLITKYPDSVKG; ó
  - CDR2 es la secuencia de aminoácido EINTNGLITKYPDSVKG; y CDR3 es la secuencia de aminoácido SPSGFN.
- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales CDR1 es la secuencia de aminoácido DYWMY; y CDR3 es la secuencia de aminoácido SPSGFN.
- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales CDR1 es la secuencia de aminoácido DYWMY; CDR2 es la secuencia de aminoácido EINTNGLITKYPDSVKG y CDR3 es la secuencia de aminoácido SPSGFN.
- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales
  - cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativa; y/o

- dicha secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácidos, y no supresiones o inserciones de aminoácidos, comparada con la(s) secuencia(s) de aminoácido(s) anteriores.

y con algunos otros aspectos particularmente preferidos siendo:

- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), el cual es un Nanocuerpo clase KERE.
- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), el cual tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en este documento) con una de las secuencias de aminoácido de las SEC ID Nos. 50 (TNF3), 83 (TNF20), 85 (TNF21), 85 (TNF22), 96 (TNF23) ó 98 (TNF33).
- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales

a) CDR1 comprende:

- la secuencia de aminoácido NYYMG; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido NYYMG; o una secuencia de aminoácido que tiene 2 ó

solamente 1 diferencia de aminoácido con la secuencia de aminoácido NYYMG;

y

b) CDR2 comprende:

- la secuencia de aminoácido NISWRGYNIYYKDSVKG; ó
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido NISWRGYNIYYKDSVKG; o
- una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido NISWRGYNIYYKDSVKG;

y

c) CDR3 comprende:

- la secuencia de aminoácido SILPLSDDPGWNTY; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido SILPLSDDPGWNTY; o
- una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia de aminoácido con la secuencia de aminoácido SILPLSDDPGWNTY.

- Un Nanocuerpo de conformidad con cualquiera de XXI)

766

hasta XXV), en los cuales CDR1 comprende la secuencia de aminoácido NYYMG.

- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales CDR2 comprende la secuencia de aminoácido NISWRGYNIYYKDSVKG.
- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales CDR3 comprende la secuencia de aminoácido SILPLSDDPGWNTY.
- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales:
  - CDR1 comprende la secuencia de aminoácido NYYMG; y CDR3 comprende la secuencia de aminoácido SILPLSDDPGWNTY; ó
  - CDR1 comprende la secuencia de aminoácido NYYMG; y CDR2 comprende la secuencia de aminoácido NISWRGYNIYYKDSVKG; ó
  - CDR2 comprende la secuencia de aminoácido NISWRGYNIYYKDSVKG; y CDR3 comprende la secuencia de aminoácido SILPLSDDPGWNTY.
- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales CDR1 comprende la secuencia de aminoácido NYYMG; CDR2 comprende la secuencia de aminoácido SILPLSDDPGWNTY y CDR3 comprende la secuencia de aminoácido ILPLSDDPGWNTY.
- Un Nanocuerpo de conformidad con cualquiera de XXI)

hasta XXV), en los cuales

a) CDR1 es:

- la secuencia de aminoácido NYYMG; o

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido NYYMG; o

- una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia de aminoácido con la secuencia de aminoácido NYYMG;

y

b) CDR2 es:

- la secuencia de aminoácido NISWRGYNIYYKDSVKG; o

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido NISWRGYNIYYKDSVKG; o

- una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido NISWRGYNIYYKDSVKG;

y

c) CDR3 es:

- la secuencia de aminoácido SILPLSDDPGWNTY; o

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido SILPLSDDPGWNTY; o

- una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia de aminoácido con la secuencia de aminoácido SILPLSDDPGWNTY.

- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales CDR1 es la secuencia de aminoácido NYYMG.

- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales CDR2 es la secuencia de aminoácido NISWRGYNIYYKDSVKG.

- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales CDR3 es la secuencia de aminoácido SILPLSDDPGWNTY.

- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales:

  - CDR1 es la secuencia de aminoácido NYYMG; y CDR3 es la secuencia de aminoácido SILPLSDDPGWNTY; o

  - CDR1 es la secuencia de aminoácido NYYMG; y CDR2 es la secuencia de aminoácido NISWRGYNIYYKDSVKG; ó

  - CDR2 es la secuencia de aminoácido NISWRGYNIYYKDSVKG; y CDR3 es la secuencia de

aminoácido SILPLSDDPGWNTY.

- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales CDR1 es la secuencia de aminoácido NYYMG; CDR2 es la secuencia de aminoácido SILPLSDDPGWNTY y CDR3 es la secuencia de aminoácido ILPLSDDPGWNTY.
- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), en los cuales cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativa; y/o
- dicha secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácidos, y no supresiones o inserciones de aminoácidos, comparada con la(s) secuencia(s) de aminoácido(s) anteriores.
- Un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV), el cual es un Nanocuerpo humanizado, y con algunos otros aspectos particularmente preferidos siendo:

XXVI) Una proteína o polipéptido, el cual comprende o consiste esencialmente de al menos un Nanocuerpo de conformidad con cualquiera de XXI) hasta XXV).

XXVII) Una proteína o polipéptido, el cual comprende dos Nanocuerpos de conformidad con cualquiera de XXI) hasta XXV).

XXVIII) Una proteína o polipéptido, el cual comprende dos

790

Nanocuerpos de conformidad con cualquiera de XXI) hasta XXV), y el cual es de manera tal que dicha proteína o polipéptido, después del enlace a un trímero de TNF, es capaz de inhibir o reducir la reticulación del receptor de TNF que es mediada por dicho trímero de TNF y/o la transducción de señal que es mediada por tal reticulación del receptor.

XXIX) Una proteína o polipéptido, el cual comprende dos Nanocuerpos de conformidad con cualquiera de XXI) hasta XXV), y el cual es capaz de enlazarse intramolecularmente con al menos dos sitios de enlace al receptor TNF en un trímero de TNF.

XXX) Una proteína o polipéptido, el cual comprende dos Nanocuerpos de conformidad con cualquiera de XXI) hasta XXV), ligados vía un enlazador adecuado.

XXXI) Una proteína o polipéptido, el cual comprende dos Nanocuerpos de conformidad con cualquiera de XXI) hasta XXV), ligados vía un enlazador adecuado, y el cual es pegilado.

XXXII) Una proteína o polipéptido el cual comprende dos Nanocuerpos de conformidad con cualquiera de XXI) hasta XXV), y el cual además, comprende al menos un Nanocuerpo dirigido contra albúmina de suero humana.

XXXIII) Una proteína o polipéptido el cual comprende dos Nanocuerpos de conformidad con cualquiera de XXI)

hasta XXV), y el cual además, comprende al menos un Nanocuerpo dirigido contra albúmina de suero humana, y el cual es de manera tal que dicha proteína o polipéptido, después del enlace a un trímero de TNF, es capaz de inhibir o reducir la reticulación del receptor de TNF que es mediada por dicho trímero de TNF y/o la transducción de señal que es mediada por tal reticulación del receptor.

XXXIV) Una proteína o polipéptido el cual comprende dos Nanocuerpos de conformidad con cualquiera de XXI) hasta XXV), y el cual además, comprende al menos un Nanocuerpo dirigido contra albúmina de suero humana y el cual es capaz de enlazarse intramolecularmente con al menos dos sitios de enlace al receptor TNF en un trímero de TNF.

XXXV) Una proteína o polipéptido el cual comprende dos Nanocuerpos de conformidad con cualquiera de XXI) hasta XXV), y el cual además, comprende un Nanocuerpo dirigido contra albúmina de suero humana, en los cuales cada uno de los dos Nanocuerpos de conformidad con cualquiera de XXI) hasta XXV) es ligado, opcionalmente vía un enlazador adecuado, a un Nanocuerpo dirigido contra albúmina de suero humana.

XXXVI) Una proteína o polipéptido el cual comprende dos Nanocuerpos de conformidad con cualquiera de XXI)

hasta XXV), y el cual además, comprende un Nanocuerpo dirigido contra albúmina de suero humana, en los cuales cada uno de los dos Nanocuerpos de conformidad con cualquiera de XXI) hasta XXV) es ligado, opcionalmente vía un enlazador adecuado, a un Nanocuerpo dirigido contra albúmina de suero humana, y el cual es de manera tal que dicha proteína o polipéptido, después del enlace a un trímero de TNF, es capaz de inhibir o reducir la reticulación del receptor de TNF que es mediada por dicho trímero de TNF y/o la transducción de señal que es mediada por tal reticulación del receptor.

XXXVII) Una proteína o polipéptido el cual comprende dos Nanocuerpos de conformidad con cualquiera de XXI) hasta XXV), y el cual además, comprende un Nanocuerpo dirigido contra albúmina de suero humana, en los cuales cada uno de los dos Nanocuerpos de conformidad con cualquiera de XXI) hasta XXV) es ligado, opcionalmente vía un enlazador adecuado, a un Nanocuerpo dirigido contra albúmina de suero humana, y el cual es capaz de enlazarse intramolecularmente con al menos dos sitios de enlace al receptor TNF en un trímero de TNF.

- Una proteína o polipéptido de conformidad con cualquiera de XXVI) hasta XXXVII), en los cuales al

menos un Nanocuerpo dirigido contra albúmina de suero humana es un Nanocuerpo humanizado.

- Una proteína o polipéptido de conformidad con cualquiera de XXVI) hasta XXXVII), en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es una variante humanizada del Nanocuerpo ALB 1 (SEC ID NO: 63).
- Una proteína o polipéptido de conformidad con cualquiera de XXVI) hasta XXXVII), en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es uno elegido del grupo que consiste de ALB 3 (SEC ID NO: 87), ALB 4 (SEC ID NO: 88), ALB 5 (SEC ID NO: 89), ALB 6 (SEC ID NO: 100), ALB 7 (SEC ID NO: 101), ALB 8 (SEC ID NO: 102) ALB 9 (SEC ID NO: 103) y ALB 10 (SEC ID NO: 104).
- Una proteína o polipéptido de conformidad con cualquiera de XXVI) hasta XXXVII), en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es ALB 8.
- Una proteína o polipéptido de conformidad con cualquiera de XXVI) hasta XXXVII), el cual comprende o consiste esencialmente de dos Nanocuerpos humanizados de conformidad con cualquiera de XXI) hasta XXV), y una variante humanizada del Nanocuerpo ALB 1 (SEC ID NO: 63).

Se debe notar que cuando un Nanocuerpo es mencionado anteriormente por estar "de conformidad con cualquiera de XXI) hasta XXV) anteriores", está al menos de conformidad con uno de XXI) hasta XXV), puede estar de conformidad con dos o más de XXI) hasta XXV), y puede también incluir uno o más de los otros aspectos que están indicados por estar "de conformidad con cualquiera de XXI) hasta XXV) anteriores. De manera similar, cuando una proteína o polipéptido es mencionado anteriormente por estar "de conformidad con cualquiera de XXVI) hasta XXXVII) anteriores", está al menos de conformidad con uno de XXVI) hasta XXXVII), puede estar de conformidad con dos o más de XXVI) hasta XXXVII), y puede también incluir uno o más de los otros aspectos que están indicados por estar "de conformidad con cualquiera de XXVI) hasta XXXVII) anteriores.

Un clon que se ha encontrado por ser particularmente útil como un Nanocuerpo anti-TNF, es el clon PMP1C2 (TNF1). Como se puede ver a partir de los datos comparativos del ensayo KYM en la Tabla 39, el TNF1 tiene un valor EC_{50} que es más de 4 veces mejor que el mejor Nanocuerpo monovalente descrito en el documento WO 04/41862 (Nanocuerpo 3E), es decir, 2,466 nM para PMP 1C2 contra 12 nM para 3E (como se puede ver de la Tabla 39, todos los Nanocuerpos TNF1 a TNF 9 de la invención, dan un mejor

valor EC50 en este ensayo que en 3E). En este sentido, se debe notar que el Nanocuerpo 3E del documento WO 04/41862, pertenece a la "clase KERE" (como se describe en este documento), y puede por lo tanto, ser humanizado a un grado menor que el Nanocuerpo PMP1C2 (el cual pertenece a la "clase GLEW"). Cuando el Nanocuerpo PMP1C2 es comparado con el Nanocuerpo IA del documento WO 04/41862, un Nanocuerpo clase GLEW con el grado más alto en homología de secuencia con PMP 1C2 (en tanto las CDR como las estructuras), el valor EC_{50} obtenido para PMP1C2 en el ensayo KYM, es más de 50 veces mejor, es decir, 2.466 nM para PMP1C2, comparado con 100 nM para IA.

Por consiguiente, Nanocuerpos que comprenden uno o más, preferiblemente cualquiera dos y más, preferiblemente tres de las CDR presentes en el clon de PMP1C2 (o secuencias CDR que son derivadas a partir de estas o correspondientes a estas), son particularmente preferidas en la invención. También, estos Nanocuerpos preferiblemente pertenecen al "grupo 103 P,R,S" (como se define en este documento), y más preferiblemente tienen una R en la posición 103, y preferiblemente también tienen una GLEW o secuencia similar a GLEW en las posiciones 44-47. También, cuando estos Nanocuerpos pertenecen al "grupo 103 P, R, S" (y en particular, cuando tienen una R en la posición 103), una substitución humanizante preferida pero

no limitante es 108Q hasta 108L. Otras sustituciones humanizantes preferidas pero no limitantes en estos Nanocuerpos preferidos, son una o más de aquellas presentes en las variantes humanizadas de TNF1 descrito en este documento, tales como TNF13, TNF14, TNF 29 ó TNF30, como será inmediatamente claro a partir de una comparación entre la secuencia de NTF1 y/o estas secuencias humanizadas.

De este modo, en un Nanocuerpo particularmente preferido de la invención, al menos una de las secuencias CDR1, CDR2 y CDR3 presente, es adecuadamente elegida del grupo que consiste de las secuencias CDR1 de la SEC ID NO: 164, la secuencia CDR2 de la SEC ID NO: 232, y la secuencia CDR3 de la SEC ID NO: 300, respectivamente (es decir, las secuencias CDR presentes en el clon TNF1), o del grupo de las secuencias CDR1, CDR2 y CDR3, respectivamente, que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de "identidad de secuencia" (como se define en este documento) con la secuencia CDR1 de la SEC ID NO: 164, la secuencia CDR2 de la SEC ID NO: 232, y la secuencia CDR3 de la SEC ID NO: 300, respectivamente; y/o del grupo que consiste de las secuencias CDR1, CDR2 y CDR3, respectivamente, que tienen 3, 2 ó solamente 1 "diferencia(s) de aminoácido(s)" (como se define en este documento) con la secuencia CDR1 de la SEC ID NO: 164, la

secuencia CDR2 de la SEC ID NO: 232, y la secuencia CDR3 de la SEC ID NO: 300, respectivamente.

Preferiblemente, en estos Nanocuerpos preferidos de la invención, al menos dos de las secuencias CDR1, CDR2 y CDR3 presentes son adecuadamente elegidas del grupo que consiste de la secuencia CDR1 de la SEC ID NO: 164, la secuencia CDR2 de la SEC ID NO: 232, y la secuencia CDR3 de la SEC ID NO: 300, respectivamente (es decir, las secuencias CDR presentes en el clon TNF1), o del grupo que consiste de secuencias CDR1, CDR2 y CDR3, respectivamente, que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia CDR1 de la SEC ID NO: 164, la secuencia CDR2 de la SEC ID NO: 232, y la secuencia CDR3 de la SEC ID NO: 300, respectivamente; y/o del grupo que consiste de las secuencias CDR1, CDR2 y CDR3, respectivamente, que tienen 3, 2 ó solamente 1 "diferencia(s) de aminoácido(s)" con la secuencia CDR1 de la SEC ID NO: 164, la secuencia CDR2 de la SEC ID NO: 232, y la secuencia CDR3 de la SEC ID NO: 300, respectivamente.

Más preferiblemente, en estos Nanocuerpos preferidos de la invención, las tres secuencias CDR1, CDR2 y CDR3 presentes son adecuadamente elegidas del grupo que consiste de la secuencia CDR1 de la SEC ID NO: 164, la

secuencia CDR2 de la SEC ID NO: 232, y la secuencia CDR3 de la SEC ID NO: 300, respectivamente (es decir, las secuencias CDR presentes en el clon TNF1), o del grupo de las secuencias CDR1, CDR2 y CDR3, respectivamente, que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia CDR1 de la SEC ID NO: 164, la secuencia CDR2 de la SEC ID NO: 232, y la secuencia CDR3 de la SEC ID NO: 300, respectivamente; y/o del grupo que consiste de las secuencias CDR1, CDR2 y CDR3, respectivamente, que tienen 3, 2 ó solamente 1 diferencia(s) de aminoácido(s) con la secuencia CDR1 de la SEC ID NO: 164, la secuencia CDR2 de la SEC ID NO: 232, y la secuencia CDR3 de la SEC ID NO: 300, respectivamente.

Aún más preferiblemente, en estos Nanocuerpos preferidos de la invención, al menos una de las secuencias CDR1, CDR2 y CDR3 presentes es adecuadamente elegida del grupo que consiste de la secuencia CDR1 de la SEC ID NO: 164, la secuencia CDR2 de la SEC ID NO: 232, y la secuencia CDR3 de la SEC ID NO: 300, respectivamente (es decir, las secuencias CDR presentes en el clon TNF1). Preferiblemente, en esta modalidad, al menos una o preferiblemente ambas de las otras dos secuencias CDR presentes, son adecuadamente elegidas de las secuencias CDR que tienen al menos 80%,

preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia CDR1 de la SEC ID NO: 164, la secuencia CDR2 de la SEC ID NO: 232, y la secuencia CDR3 de SEC ID NO: 300, respectivamente; y/o adecuadamente elegidas del grupo que consiste de las secuencias CDR que tienen 3, 2 ó solamente 1 diferencia(s) de aminoácido(s) con la secuencia CDR1 de la SEC ID NO: 164, la secuencia CDR2 de la SEC ID NO: 232, y la secuencia CDR3 de la SEC ID NO: 300, respectivamente.

Aún más preferiblemente, en estos Nanocuerpos preferidos de la invención, al menos dos de las secuencias CDR1, CDR2 y CDR3 presentes son adecuadamente elegidas del grupo que consiste de la secuencia CDR1 de la SEC ID NO: 164, la secuencia CDR2 de la SEC ID NO: 232, y la secuencia CDR3 de la SEC ID NO: 300, respectivamente (es decir, las secuencias CDR presentes en el clon TNF1). Preferiblemente, en esta modalidad, la secuencia CDR restante presente es adecuadamente elegida del grupo de secuencias CDR que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia CDR1 de la SEC ID NO: 164, la secuencia CDR2 de la SEC ID NO: 232, y la secuencia CDR3 de la SEC ID NO: 300, respectivamente; y/o del grupo que consiste de secuencias

CDR que tienen 3, 2 ó solamente 1 diferencia(s) de aminoácido(s) con la secuencia CDR1 de la SEC ID NO: 164, la secuencia CDR2 de la SEC ID NO: 232, y la secuencia CDR3 de la SEC ID NO: 300, respectivamente.

Nanocuerpos particularmente preferidos de la invención, comprenden la secuencia CDR1 de la SEC ID NO: 164, la secuencia CDR2 de la SEC ID NO: 232, y la secuencia CDR3 de la SEC ID NO: 300, respectivamente (es decir, las secuencias CDR presentes en el clon TNF1).

Nanocuerpos con las secuencias CDR anteriores, preferiblemente tienen secuencias de estructura que son como se definen adicionalmente en este documento. Algunas combinaciones particularmente preferidas, pero no limitantes de las secuencias de estructura, se pueden ver en las Tablas I anteriores. Como será claro para la persona experta, una combinación de secuencias FR1, FR2, FR3 y FR4 que ocurre en el mismo clon (es decir, secuencias FR1, FR2, FR3 y FR4 las cuales son mencionadas en la misma línea en la Tabla I), usualmente serán preferidas (aunque la invención en su sentido más amplio no está limitada a esta, y también comprende otras combinaciones adecuadas de las secuencias de estructura mencionadas en la Tabla I).

Más específicamente, algunos aspectos preferidos de esta modalidad de la invención son:

XXXVIII) Un Nanocuerpo contra TNF-alfa, el cual consiste

de 4 regiones de estructura (FR1 hasta FR4 respectivamente) y 3 regiones que determinan la complementariedad (CDR1 hasta CDR3 respectivamente), en las cuales:

a) CDR1 comprende:

- la secuencia de aminoácido DYWMY; ó

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido DYWMY; o

- una secuencia de aminoácido que tiene solamente 1 diferencia de aminoácido con la secuencia de aminoácido DYWMY;

y

b) CDR2 comprende:

- la secuencia de aminoácido EINTNGLITKYPDSVKG; o una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido EINTNGLITKYPDSVKG; o

- una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido EINTNGLITKYPDSVKG;

y

c) CDR3 comprende:

- la secuencia de aminoácido SPSGFN; o

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido SPSGFN; o

- una secuencia de aminoácido que tiene solamente 1 diferencia de aminoácido con la secuencia de aminoácido SPSGFN.

- Un Nanocuerpo de conformidad con XXXVIII), en los cuales CDR1 comprende la secuencia de aminoácido DYWMY.

- Un Nanocuerpo de conformidad con XXXVIII), en los cuales CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG.

- Un Nanocuerpo de conformidad con XXXVIII), en los cuales CDR3 comprende la secuencia de aminoácido SPSGFN.

- Un Nanocuerpo de conformidad con XXXVIII), en los cuales:

- CDR1 comprende la secuencia de aminoácido DYWMY; y CDR3 comprende la secuencia de aminoácido SPSGFN; o

- CDR1 comprende la secuencia de aminoácido DYWMY; y

CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG; ó

- CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG; y CDR3 comprende la secuencia de aminoácido SPSGFN.
- Un Nanocuerpo de conformidad con XXXVIII), en los cuales CDR1 comprende la secuencia de aminoácido DYWMY; y CDR3 comprende la secuencia de aminoácido SPSGFN.
- Un Nanocuerpo de conformidad con XXXVIII) en los cuales CDR1 comprende la secuencia de aminoácido DYWMY; CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG y CDR3 comprende la secuencia de aminoácido SPSGFN.
- Un Nanocuerpo de conformidad con XXXVIII), en los cuales,
  - cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativa; y/o
  - dicha secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácidos, y no supresiones o inserciones de aminoácidos, comparada con la(s) secuencia(s) de aminoácido(s) anteriores.
- Un Nanocuerpo de conformidad con XXXVIII), el cual es un Nanocuerpo clase GLEW.
- Un Nanocuerpo de conformidad con XXXVIII), el cual

contiene un residuo de arginina (R) en la posición 103.

- Un Nanocuerpo de conformidad con XXXVIII), el cual tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en este documento) con una de las secuencias de aminoácido de las SEC ID Nos. 52 (TNF1), 76 (TNF13), 77 (TNF14), 95 (TNF29) ó 96 (TNF30).
- Un Nanocuerpo de conformidad con XXXVIII), el cual es un Nanocuerpo humanizado.
- Un Nanocuerpo de conformidad con XXXVIII), el cual contiene un residuo leucina (L) en la posición 108.
- Un Nanocuerpo de conformidad con XXXVIII), el cual tiene una relación Koff para TNF mejor que 2.10-3 2.10-3 (1/s), preferiblemente mejor que 1.10-3 (1/s); o una variante humanizada de tal Nanocuerpo;
- Un Nanocuerpo de conformidad con XXXVIII), el cual tiene un valor EC50 en el ensayo a base de célula usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que el valor EC50 del Nanocuerpo VHH 3E (SEC ID NO:4) del documento WO 04/041862 en el mismo ensayo; o una variante humanizada de tal Nanocuerpo.
- Un Nanocuerpo de conformidad con XXXVIII), el cual

tiene un valor EC50 en el ensayo a base de célula usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que 5nM; o una variante humanizada de tal Nanocuerpo.

- Un Nanocuerpo de conformidad con XXXVIII), el cual tiene un valor EC50 en el ensayo a base de célula usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que 3nM; o una variante humanizada de tal Nanocuerpo.

XXXIX) Un Nanocuerpo contra TNF-alfa, el cual consiste de 4 regiones de estructura (FR1 hasta FR4 respectivamente) y 3 regiones que determinan la complementariedad (CDR1 hasta CDR3 respectivamente), en las cuales

a) CDR1 es:

- la secuencia de aminoácido DYWMY; o

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido DYWMY; o

- una secuencia de aminoácido que tiene solamente 1 diferencia de aminoácido con la secuencia de aminoácido DYWMY;

y en las cuales:

b) CDR2 es:

- la secuencia de aminoácido EINTNGLITKYPDSVKG; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido EINTNGLITKYPDSVKG; o
- una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido EINTNGLITKYPDSVKG;

y en las cuales

c) CDR3 es:

- la secuencia de aminoácido SPSGFN; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido SPSGFN; o
- una secuencia de aminoácido que tiene solamente 1 diferencia de aminoácido con la secuencia de aminoácido SPSGFN.

- Un Nanocuerpo de conformidad con XXXIX), en los cuales CDR1 es la secuencia de aminoácido DYWMY.
- Un Nanocuerpo de conformidad con XXXIX), en los cuales CDR2 es la secuencia de aminoácido EINTNGLITKYPDSVKG.

- Un Nanocuerpo de conformidad con XXXIX), en los cuales CDR3 es la secuencia de aminoácido SPSGFN
- Un Nanocuerpo de conformidad con XXXIX), en los cuales: CDR1 es la secuencia de aminoácido DYWMY; y CDR3 es la secuencia de aminoácido SPSGFN; o
  - CDR1 es la secuencia de aminoácido DYWMY; y CDR2 es la secuencia de aminoácido EINTNGLITKYPDSVKG; ó
  - CDR2 es la secuencia de aminoácido EINTNGLITKYPDSVKG; y CDR3 es la secuencia de aminoácido SPSGFN.
- Un Nanocuerpo de conformidad con XXXIX), en los cuales CDR1 es la secuencia de aminoácido DYWMY; y CDR3 es la secuencia de aminoácido SPSGFN.
- Un Nanocuerpo de conformidad con XXXIX), en los cuales CDR1 es la secuencia de aminoácido DYWMY; CDR2 es la secuencia de aminoácido EINTNGLITKYPDSVKG y CDR3 es la secuencia de aminoácido SPSGFN.
- Un Nanocuerpo de conformidad con XXXIX), en los cuales:
  - cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativa; y/o
  - dicha secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácidos, y no supresiones o inserciones de aminoácidos, comparada con la(s) secuencia(s) de aminoácido(s) anteriores.

- Un Nanocuerpo de conformidad con XXXIX), el cual es un Nanocuerpo clase GLEW.
- Un Nanocuerpo de conformidad con XXXIX), el cual contiene un residuo de arginina (R) en la posición 103.
- Un Nanocuerpo de conformidad con XXXIX), el cual tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en este documento) con una de las secuencias de aminoácido de las SEC ID Nos. 52 (TNF1), 76 (TNF13), 77 (TNF14), 95 (TNF29) ó 96 (TNF30).
- Un Nanocuerpo de conformidad con XXXIX), el cual es un Nanocuerpo humanizado.
- Un Nanocuerpo de conformidad con XXXIX), el cual contiene un residuo leucina (L) en la posición 108.
- Un Nanocuerpo de conformidad con XXXIX), el cual tiene una relación Koff para TNF mejor de 2.10-3 (1/s), preferiblemente mejor que 1.10-3 (1/s); o una variante humanizada de tal Nanocuerpo.
- Un Nanocuerpo de conformidad con XXXIX), el cual tiene un valor EC50 en el ensayo a base de célula usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que el valor EC50 del Nanocuerpo VHH 3E (SEC ID NO:4) del documento WO

04/041862 en el mismo ensayo o una variante humanizada de tal Nanocuerpo.

- Un Nanocuerpo de conformidad con XXXIX), el cual tiene un valor EC50 en el ensayo a base de célula usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que 5nM; o una variante humanizada de tal Nanocuerpo.
- Un Nanocuerpo de conformidad con XXXIX), el cual tiene un valor EC50 en el ensayo a base de célula usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que 3nM; o una variante humanizada de tal Nanocuerpo.
- Un Nanocuerpo de conformidad con XXXIX), el cual es elegido del grupo que consiste de TNF 13 (SEC ID NO: 76), TNF 14 (SEC ID NO: 77), TNF 29 (SEC ID NO: 95) y TNF 30 (SEC ID NO:96).
- Un Nanocuerpo de conformidad con XXXIX), el cual es TNF 30 (SEC ID NO: 96); con algunos otros aspectos siendo:

XL) Una proteína o polipéptido, el cual comprende o consiste esencialmente de un Nanocuerpo de conformidad con XXXVIII) ó XXXIX).

XLI) Una proteína o polipéptido, el cual comprende o consiste esencialmente de al menos un Nanocuerpo de conformidad con XXXVIII) ó XXXIX).

- Una proteína o polipéptido de conformidad con

cualquiera de XL) ó XLI), el cual comprende dos Nanocuerpos de conformidad con XXXVIII) ó XXXIX).

- Una proteína o polipéptido de conformidad con cualquiera de XL) ó XLI), el cual comprende dos Nanocuerpos de conformidad con XXXVIII) ó XXXIX), y el cual es de manera tal que dicha proteína o polipéptido, después del enlace a un trímero de TNF, es capaz de inhibir o reducir la reticulación del receptor de TNF que es mediada por dicho trímero de TNF y/o la transducción de señal que es mediada por tal reticulación del receptor.
- Una proteína o polipéptido de conformidad con cualquiera de XL) ó XLI), el cual comprende dos Nanocuerpos de conformidad con XXXVIII) ó XXXIX), y el cual es capaz de enlazarse intramolecularmente con al menos dos sitios de enlace al receptor TNF en un trímero de TNF.
- Una proteína o polipéptido de conformidad con cualquiera de XL) ó XLI), el cual comprende dos Nanocuerpos de conformidad con XXXVIII) ó XXXIX), los cuales son directamente ligados entre sí ó enlazados entre sí vía un enlazador.
- Una proteína o polipéptido de conformidad con cualquiera de XL) ó XLI), el cual comprende dos Nanocuerpos de conformidad con XXXVIII) ó XXXIX), los

cuales son ligados entre sí vía una secuencia de aminoácido.

- Una proteína o polipéptido de conformidad con cualquiera de XL) ó XLI), el cual comprende dos Nanocuerpos de conformidad con XXXVIII) ó XXXIX), los cuales son ligados entre sí vía una secuencia de aminoácido (tal como, sin limitación, una secuencia de aminoácido que comprende residuos de glicina y serina), que comprende al menos 14 aminoácidos, más preferiblemente al menos 17 aminoácidos, tal como aproximadamente 20-40 aminoácidos (tal como el enlazador GS30).
- Una proteína o polipéptido de conformidad con cualquiera de XL) ó XLI), el cual comprende o consiste esencialmente del polipéptido TNF 7 (SEC ID NO: 73), en los cuales ambos Nanocuerpos TNF 1 han sido humanizados.
- Una proteína o polipéptido de conformidad con cualquiera de XL) ó XLI), el cual comprende o consiste esencialmente del polipéptido TNF 55 (SEC ID NO: 419) ó TNF 56 (SEC ID NO: 420).
- Una proteína o polipéptido de conformidad con cualquiera de XL) ó XLI), el cual es pegilado.
- Una proteína o polipéptido de conformidad con cualquiera de XL) ó XLI), el cual comprende dos

Nanocuerpos de conformidad con XXXVIII) ó XXXIX), y el cual es de manera tal que dicha proteína o polipéptido, después del enlace a un trímero de TNF, es capaz de inhibir o reducir la reticulación del receptor de TNF que es mediada por dicho trímero de TNF y/o la transducción de señal que es mediada por tal reticulación del receptor; y/o el cual es de manera tal que dicha proteína o polipéptido es capaz de enlazarse intramolecularmente con al menos dos sitios de enlace al receptor TNF en un trímero de TNF, y en el cual la proteína o polipéptido además comprende al menos un Nanocuerpo dirigido contra albúmina de suero humana.

- Una proteína o polipéptido de conformidad con cualquiera de XL) ó XLI), el cual comprende dos Nanocuerpos de conformidad con XXXVIII) ó XXXIX), y en el cual la proteína o polipéptido además comprende al menos un Nanocuerpo dirigido contra albúmina de suero humana, en los cuales los dos Nanocuerpos de conformidad con XXXVIII) o XXXIX) están ligados entre sí vía al menos un Nanocuerpo dirigido contra albúmina de suero humana, y en los cuales los dos Nanocuerpos de conformidad con XXXVIII) ó XXXIX) están ya sea ligados directamente con al menos un Nanocuerpo dirigido contra albúmina de suero humana, ó están

ligados con al menos un Nanocuerpo dirigido contra albúmina de suero humana vía un enlazador.

- Una proteína o polipéptido de conformidad con cualquiera de XL) ó XLI), el cual comprende dos Nanocuerpos de conformidad con XXXVIII) ó XXXIX), y en el cual la proteína o polipéptido además comprende al menos un Nanocuerpo dirigido contra albúmina de suero humana, en el cual los dos Nanocuerpos de conformidad con XXXVIII) ó XXXIX) están ligados entre sí vía al menos un Nanocuerpo dirigido contra albúmina de suero humana, y en los cuales los dos Nanocuerpos de conformidad con XXXVIII) ó XXXIX) están ya sea ligados directamente con al menos un Nanocuerpo dirigido contra albúmina de suero humana, ó están ligados con al menos un Nanocuerpo dirigido contra albúmina de suero humana vía un enlazador, en los cuales el enlazador es una secuencia de aminoácido (tal como, sin limitación, un enlazador que comprende residuos de glicina y serina), y en particular una secuencia de aminoácido que comprende entre 3 y 40 residuos de aminoácidos, tal como entre 5 y 15 residuos de aminoácidos (tal como el enlazador GS9).
- Una proteína o polipéptido de conformidad con cualquiera de XL) ó XLI), el cual comprende dos Nanocuerpos de conformidad con XXXVIII) ó XXXIX), y en

el cual la proteína o polipéptido además comprende al menos un Nanocuerpo dirigido contra albúmina de suero humana, en los cuales los dos Nanocuerpos de conformidad con XXXVIII) ó XXXIX) están ligados entre sí vía al menos un Nanocuerpo dirigido contra albúmina de suero humana, y en los cuales los dos Nanocuerpos de conformidad con XXXVIII) ó XXXIX) están ya sea ligados directamente con al menos un Nanocuerpo dirigido contra albúmina de suero humana, ó están ligados con al menos un Nanocuerpo dirigido contra albúmina de suero humana vía un enlazador, y en los cuales la proteína o polipéptido es de manera tal que dicha proteína o polipéptido, después del enlace a un trímero de TNF, es capaz de inhibir o reducir la reticulación del receptor de TNF que es mediada por dicho trímero de TNF y/o la transducción de señal que es mediada por tal reticulación del receptor; y/o en los cuales la proteína o polipéptido es de manera tal que dicha proteína o polipéptido es capaz de enlazarse intramolecularmente con al menos dos sitios de enlace al receptor TNF en un trímero de TNF.

- Una proteína o polipéptido de conformidad con cualquiera de XL) ó XLI), en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es un Nanocuerpos humanizado.

- Una proteína o polipéptido de conformidad con cualquiera de XL) ó XLI), en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es una variante humanizada del Nanocuerpo ALB 1 (SEC ID NO: 63).
- Una proteína o polipéptido de conformidad con cualquiera de XL) ó XLI), en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es uno elegido del grupo que consiste de ALB 3 (SEC ID NO: 87), ALB 4 (SEC ID NO: 88), ALB 5 (SEC ID NO: 89), ALB 6 (SEC ID NO: 100), ALB 7 (SEC ID NO: 101), ALB 8 (SEC ID NO: 102) ALB 9 (SEC ID NO: 103) y ALB 10 (SEC ID NO: 104).
- Una proteína o polipéptido de conformidad con cualquiera de XL) ó XLI), en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es ALB 8.
- Una proteína o polipéptido de conformidad con cualquiera de XL) ó XLI), el cual comprende o consiste esencialmente de dos Nanocuerpos humanizados de conformidad con cualquiera de XL) ó XLI) y una variante humanizada del Nanocuerpo ALB 1 (SEC ID NO: 63).
- Una proteína o polipéptido de conformidad con cualquiera de XL) ó XLI), el cual comprende o consiste

esencialmente del polipéptido TNF24 (SEC ID NO: 90), en los cuales tanto el Nanocuerpo TNF1, así como también el Nanocuerpo ALB 1, han sido humanizados.

- Una proteína o polipéptido de conformidad con cualquiera de XL) ó XLI), el cual comprende o consiste esencialmente de dos Nanocuerpos TNF 30 y un Nanocuerpo ALB 8.
- Una proteína o polipéptido de conformidad con cualquiera de XL) ó XLI), el cual comprende o consiste esencialmente del polipéptido TNF 60 (SEC ID NO: 417).

Se debe notar que cuando un Nanocuerpo es mencionado anteriormente por estar "de conformidad con XXXVIII" o "de conformidad con XXXIX", está al menos de conformidad con uno de XXXVIII) y/o XXXIX), y puede también incluir uno o más de los otros aspectos que están indicados por estar "de conformidad con XXXVIII" o "de conformidad con XXXIX" anteriores. De manera similar, cuando una proteína o polipéptido es mencionado anteriormente por estar "de conformidad con cualquiera de XL) ó XLI)", está al menos de conformidad con uno de XL) hasta XLI), puede estar de conformidad con dos o más de VI) hasta XVIII), y puede también incluye cualquiera o más de los otros aspectos que están indicados por estar "de conformidad con cualquiera de XL) ó XLI) anteriores.

Para los Nanocuerpos basados en el Nanocuerpos

TNF1 anterior (que incluye pero no se limita a los Nanocuerpos humanizados), las secuencias de estructura pueden en general, ser como se describen en este documento, y preferiblemente, son como siguen:

a) FR1 comprende o es:

- la secuencia de aminoácido de la SEC ID NO: 130; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido de la SEC ID NO: 130; o
- una secuencia de aminoácido que tiene solamente 1 diferencia de aminoácido con la secuencia de aminoácido de la SEC ID NO: 130;

y

b) FR2 comprende o es:

- la secuencia de aminoácido de la SEC ID NO: 198; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido de la SEC ID NO: 198; o una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido de la SEC ID NO: 198;

y

c) FR3 comprende o es:

- la secuencia de aminoácido de la SEC ID NO: 266; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido de la SEC ID NO: 266; o
- una secuencia de aminoácido que tiene solamente 1 diferencia de aminoácido con la secuencia de aminoácido de la SEC ID NO: 266.

y

d) FR4 comprende o es:

- la secuencia de aminoácido de la SEC ID NO: 334; ó
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido de la SEC ID NO: 334; o
- una secuencia de aminoácido que tiene solamente 1 diferencia de aminoácido con la secuencia de aminoácido de la SEC ID NO: 334;

en las cuales las diferencias de aminoácidos presentes en las secuencias de estructura son más preferiblemente, como se describe en este documento.

Los Nanocuerpos contra TNF, los cuales tienen regiones de estructura como se describe anteriormente (es decir, similar a TNF1), y en los cuales al menos una de las regiones de estructura (tal como alguna de dos, alguna de tres o las cuatro regiones de estructura) han sido humanizadas, forman un aspecto adicional de la invención. Tales Nanocuerpos pueden en particular, tener CDR que son tales que el Nanocuerpo tiene una relación Koff para TNF mejor de 2.10-3 (1/s), preferiblemente mejor que 1.10-3 (1/s); y/o tienen CDR que son de manera tal que el Nanocuerpo tiene un valor EC50 en el ensayo a base de célula usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que el valor EC50 del Nanocuerpo VHH 3E (SEC ID NO:4) del documento WO 04/041862 en el mismo ensayo; y en particular mejor que 12nM, más en particular mejor que 5 nM, aún más en particular mejor que 3nM. También, ó alternativamente, tales Nanocuerpos son preferiblemente dirigidos contra el mismo epítope de TNF (es decir, el trímero de TNF) como TNF1.

En particular, la invención se refiere a un Nanocuerpo contra TNF-alfa, el cual es una variante humanizada de un Nanocuerpo contra TNF-alfa, en el cual, el Nanocuerpo contra TNF-alfa tiene las siguientes secuencias de estructura: FR1: SEC ID NO: 130; FR2: SEC ID

NO: 198; FR3: SEC ID NO: 266; y FR4: SEC ID NO: 334. Tal nanocuerpo puede en particular, tener CDR que son de manera tal que el Nanocuerpo tiene una relación Koff para TNF mejor de 2.10-3 (1/s), preferiblemente mejor que 1.10-3 (1/s); y/o tiene CDR que son de manera tal que el Nanocuerpo tiene un valor EC50 en el ensayo a base de células usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que el valor EC50 del Nanocuerpo VHH 3E (SEC ID NO:4) del documento WO 04/041862 en el mismo ensayo; y en particular mejor que 12nM, más en particular mejor que 5 nM, aún más en particular mejor que 3nM. También, ó alternativamente, tales Nanocuerpos son preferiblemente dirigidos contra el mismo epítope de TNF (es decir, el trímero de TNF) como TNF1.

Otro clon que ha sido encontrado por ser particularmente útil como un Nanocuerpo anti-TNF es el clon PMP5F10 (TNF3, SEC ID NO: 60). Como se puede ver a partir de los datos comparativos del ensayo KYM en la Tabla 39, el TNF3 tiene un valor EC50 que es más de 15 veces mejor que el mejor Nanocuerpo monovalente descrito en el documento WO 04/41862.

Por consiguiente, los Nanocuerpos que comprenden uno o más, preferiblemente cualquiera dos y más preferiblemente las tres CDR presentes en el clon PMP5F10

(o secuencias CDR que son derivadas a partir de estas o corresponden a estas) son particularmente preferidas en la invención. También, estos Nanocuerpos preferiblemente pertenecen a la clase KERE.

Más específicamente, algunos aspectos preferidos de esta modalidad de la invención son:

XLII) Un Nanocuerpo contra TNF-alfa, el cual consiste de 4 regiones de estructura (FR1 hasta FR4 respectivamente) y 3 regiones que determinan la complementariedad (CDR1 hasta CDR3 respectivamente), en los cuales

a) CDR1 comprende:

- la secuencia de aminoácido NYYMG; o
- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido NYYMG; o
- una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia de aminoácido con la secuencia de aminoácido NYYMG;

Y

b) CDR2 comprende:

- la secuencia de aminoácido NISWRGYNIYYKDSVKG; o
- una secuencia de aminoácido que tiene al menos 80%,

preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido NISWRGYNIYYKDSVKG; o

- una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido NISWRGYNIYYKDSVKG;

y

c) CDR3 comprende:

- la secuencia de aminoácido SILPLSDDPGWNTY; o

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido SILPLSDDPGWNTY; ó

- una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia de aminoácido con la secuencia de aminoácido SILPLSDDPGWNTY.

- Un Nanocuerpo de conformidad con XLII), en los cuales CDR1 comprende la secuencia de aminoácido NYYMG.

- Un Nanocuerpo de conformidad con XLII), en los cuales CDR2 comprende la secuencia de aminoácido NISWRGYNIYYKDSVKG.

- Un Nanocuerpo de conformidad con XLII), en los cuales CDR3 comprende la secuencia de aminoácido

SILPLSDDPGWNTY.

- Un Nanocuerpo de conformidad con XLII), en los cuales:
  - CDR1 comprende la secuencia de aminoácido NYYMG; y CDR3 comprende la secuencia de aminoácido SILPLSDDPGWNTY; o
  - CDR1 comprende la secuencia de aminoácido NYYMG; y CDR2 comprende la secuencia de aminoácido NISWRGYNIYYKDSVKG; o
  - CDR2 comprende la secuencia de aminoácido NISWRGYNIYYKDSVKG; y CDR3 comprende la secuencia de aminoácido SILPLSDDPGWNTY.
- Un Nanocuerpo de conformidad con XLII), en los cuales CDR1 comprende la secuencia de aminoácido NYYMG; CDR2 comprende la secuencia de aminoácido SILPLSDDPGWNTY y CDR3 comprende la secuencia de aminoácido ILPLSDDPGWNTY.
- Un Nanocuerpo de conformidad con XLII), en los cuales
  - cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativa; y/o
  - dicha secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácidos, y no supresiones o inserciones de aminoácidos, comparada con la(s) secuencia(s) de aminoácido(s) anteriores.
- Un Nanocuerpo de conformidad con XLII), el cual es un

Nanocuerpo clase KERE.

- Un Nanocuerpo de conformidad con XLII), el cual tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en este documento) con una de las secuencias de aminoácido de las SEC ID Nos. 50 (TNF3), 83 (TNF20), 85 (TNF21), 85 (TNF22), 96 (TNF23) ó 99 (TNF33).
- Un Nanocuerpo de conformidad con XLII), el cual es un Nanocuerpo humanizado.
- Un Nanocuerpo de conformidad con XLII), el cual tiene una relación Koff para TNF mejor de 2.10^{-3} (1/s), preferiblemente mejor que 1.10^{-3} (1/s), ó una variante humanizada de tal Nanocuerpo.
- Un Nanocuerpo de conformidad con XLII), el cual tiene un valor EC50 en el ensayo a base de células usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que el valor EC50 del Nanocuerpo VHH 3E (SEC ID NO 4) del documento WO 04/041862 en el mismo ensayo, o una variante humanizada de tal Nanocuerpo.
- Un Nanocuerpo de conformidad con XLII), el cual tiene un valor EC50 en el ensayo a base de células usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que 5nM, ó una

variante humanizada de tal Nanocuerpo.

- Un Nanocuerpo de conformidad con XLII), el cual tiene un valor EC50 en el ensayo a base de célula, usando células KYM descritas in Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que 3nM, ó una variante humanizada de tal Nanocuerpo.

XLIII) Un Nanocuerpo contra TNF-alfa, el cual consiste de 4 regiones de estructura (FR1 hasta FR4 respectivamente) y 3 regiones que determinan la complementariedad (CDR1 hasta CDR3 respectivamente), en las cuales

a) CDR1 es:

- la secuencia de aminoácido NYYMG, o

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido NYYMG, ó una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia de aminoácido con la secuencia de aminoácido NYYMG,

y

b) CDR2 es:

- la secuencia de aminoácido NISWRGYNIYYKDSVKG, o

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al

menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido NISWRGYNIYYKDSVKG, ó

- una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido NISWRGYNIYYKDSVKG, y

c) CDR3 es:

- la secuencia de aminoácido SILPLSDDPGWNTY; o

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido SILPLSDDPGWNTY; o

- una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia de aminoácido con la secuencia de aminoácido SILPLSDDPGWNTY.

- Un Nanocuerpo de conformidad con XLIII), en los cuales CDR1 es la secuencia de aminoácido NYYMG.

- Un Nanocuerpo de conformidad con XLIII), en los cuales CDR2 es la secuencia de aminoácido NISWRGYNIYYKDSVKG.

- Un Nanocuerpo de conformidad con XLIII), en los cuales CDR3 es la secuencia de aminoácido SILPLSDDPGWNTY.

- Un Nanocuerpo de conformidad con XLIII), en los cuales:

- CDR1 es la secuencia de aminoácido NYYMG; y CDR3 es la secuencia de aminoácido SILPLSDDPGWNTY; o

- CDR1 es la secuencia de aminoácido NYYMG; y CDR2 es la secuencia de aminoácido NISWRGYNIYYKDSVKG; ó

- CDR2 es la secuencia de aminoácido NISWRGYNIYYKDSVKG; y CDR3 es la secuencia de aminoácido SILPLSDDPGWNTY.

- Un Nanocuerpo de conformidad con XLIII), en los cuales CDR1 es la secuencia de aminoácido NYYMG; CDR2 es la secuencia de aminoácido SILPLSDDPGWNTY y CDR3 es la secuencia de aminoácido ILPLSDDPGWNTY.

- Un Nanocuerpo de conformidad con XLIII), en los cuales

- cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativa; y/o

- dicha secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácidos, y no supresiones o inserciones de aminoácidos, comparada con la(s) secuencia(s) de aminoácido(s) anteriores.

- Un Nanocuerpo de conformidad con XLIII), el cual es un Nanocuerpo clase KERE.

- Un Nanocuerpo de conformidad con XLIII), el cual tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en este documento) con una de las secuencias de aminoácido de las SEC ID Nos. 50 (TNF3), 83 (TNF20),

85 (TNF21), 85 (TNF22), 96 (TNF23) ó 99 (TNF33).

- Un Nanocuerpo de conformidad con XLIII), el cual es un Nanocuerpo humanizado.
- Un Nanocuerpo de conformidad con XLIII), el cual tiene una relación Koff para TNF mejor de 2.10^{-3} (1/s), preferiblemente mejor que 2.10^{-3} (1/s); o una variante humanizada de tal Nanocuerpo.
- Un Nanocuerpo de conformidad con XLIII), el cual tiene un valor EC50 en el ensayo a base de células usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que el valor EC50 del Nanocuerpo VHH 3E (SEC ID NO:4) del documento WO 04/041862 en el mismo ensayo; o una variante humanizada de tal Nanocuerpo.
- Un Nanocuerpo de conformidad con XLIII), el cual tiene un valor EC50 en el ensayo a base de células usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que 5nM; o una variante humanizada de tal Nanocuerpo.
- Un Nanocuerpo de conformidad con XLIII), el cual tiene un valor EC50 en el ensayo a base de células usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que 3nM; o una variante humanizada de tal Nanocuerpo.
- Un Nanocuerpo de conformidad con XLIII), el cual es

elegido del grupo que consiste de las SEC ID Nos., 83 (TNF20), 85 (TNF21), 85 (TNF22), 96 (TNF23) ó 98 (TNF33) TNF 13 (SEC ID NO: 76), TNF 14 (SEC ID NO: 77), TNF 29 (SEC ID NO: 95) y TNF 30 (SEC ID NO:96),

con algunos otros aspectos siendo:

XLIV) Una proteína o polipéptido, el cual comprende o consiste esencialmente de un Nanocuerpo de conformidad con XLII) ó XLIII).

XLV) Una proteína o polipéptido, el cual comprende o consiste esencialmente de al menos un Nanocuerpo de conformidad con XLII) ó XLIII).

XLVI) Una proteína o polipéptido, el cual comprende dos Nanocuerpos de conformidad con XLII) ó XLIII).

XLVII) Una proteína o polipéptido, el cual comprende dos Nanocuerpos de conformidad con XLII) ó XLIII), y el cual es de manera tal que dicha proteína o polipéptido, después del enlace a un trímero de TNF, es capaz de inhibir o reducir la reticulación del receptor de TNF que es mediada por dicho trímero de TNF y/o la transducción de señal que es mediada por tal reticulación del receptor.

XLVIII) Una proteína o polipéptido, el cual comprende dos Nanocuerpos de conformidad con XLII) ó XLIII), y el cual es capaz de enlazarse intramolecularmente con al menos dos sitios de enlace al receptor TNF en un

trímero de TNF.

- Una proteína o polipéptido de conformidad con cualquiera de XLIV) ó XLVIII), el cual comprende o consiste esencialmente del polipéptido TNF 6 (SEC ID NO: 72) ó TNF 9 (SEC ID NO: 75, en los cuales ambos Nanocuerpos TNF 3 han sido humanizados.
- Una proteína o polipéptido de conformidad con cualquiera de XLIV) ó XLVIII), el cual es pegilado.
- Una proteína o polipéptido, el cual comprende dos Nanocuerpos de conformidad con XLII) ó XLIII), y el cual es de manera tal que dicha proteína o polipéptido, después del enlace a un trímero de TNF, es capaz de inhibir o reducir la reticulación del receptor de TNF que es mediada por dicho trímero de TNF y/o la transducción de señal que es mediada por tal reticulación del receptor; y/o el cual es de manera tal que dicha proteína o polipéptido es capaz de enlazarse intramolecularmente con al menos dos sitios de enlace al receptor TNF en un trímero de TNF, y en el cual la proteína o polipéptido además comprende al menos un Nanocuerpo dirigido contra albúmina de suero humana.
- Una proteína o polipéptido de conformidad con cualquiera de XLIV) ó XLVIII), en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana

es un Nanocuerpo humanizado.

- Una proteína o polipéptido de conformidad con cualquiera de XLIV) ó XLVIII), en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es una variante humanizada del Nanocuerpo ALB 1 (SEC ID NO: 63).
- Una proteína o polipéptido de conformidad con cualquiera de XLIV) ó XLVIII), en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es uno elegido del grupo que consiste de ALB 3 (SEC ID NO: 87), ALB 4 (SEC ID NO: 88), ALB 5 (SEC ID NO: 89), ALB 6 (SEC ID NO: 100), ALB 7 (SEC ID NO: 101), ALB 8 (SEC ID NO: 102) ALB 9 (SEC ID NO: 103) y ALB 10 (SEC ID NO: 104).
- Una proteína o polipéptido de conformidad con cualquiera de XLIV) ó XLVIII), en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es ALB 8.
- Una proteína o polipéptido de conformidad con cualquiera de XLIV) ó XLVIII), el cual comprende o consiste esencialmente de dos Nanocuerpos humanizados de conformidad con cualquiera de XLIV) ó XLVIII) y una variante humanizada del Nanocuerpo ALB 1 (SEC ID NO: 63).
- Una proteína o polipéptido de conformidad con

cualquiera de XLIV) ó XLVIII), el cual comprende ó consiste esencialmente del polipéptido TNF26 (SEC ID NO: 92), en los cuales ambos Nanocuerpos TNF 3 así como también el Nanocuerpo ALB 1 ha sido humanizado.

Se debe notar que cuando un Nanocuerpo es mencionado anteriormente por estar "de conformidad con XLII" o "de conformidad con XLIII", está al menos de conformidad con uno de XLII) y/o XLIII), y puede también incluir uno o más de los otros aspectos que están indicados por estar "de conformidad con XLII)" o "de conformidad con XLIII)" anteriores. De manera similar, cuando una proteína o polipéptido es mencionado anteriormente por estar "de conformidad con cualquiera de XLIV) ó XLVIII)", está al menos de conformidad con uno de XL) hasta XLI), puede estar de conformidad con dos o más de XLIV) hasta XLVIII), y puede también incluir uno o más de los otros aspectos que están indicados por estar "de conformidad con cualquiera de XLIV) ó XLVIII) anteriores.

Para los Nanocuerpos basados en el Nanocuerpo TNF3 anterior (que incluye pero no se limita a los Nanocuerpos humanizados), las secuencias de estructura pueden en general, ser como se describen en este documento, y preferiblemente son como sigue:

a) FR1 comprende o es:

- la secuencia de aminoácido de la SEC ID NO: 138; o

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido de la SEC ID NO: 138; o

- una secuencia de aminoácido que tiene solamente 1 diferencia de aminoácido con la secuencia de aminoácido de la SEC ID NO: 138;

y

b) FR2 comprende o es:

- la secuencia de aminoácido de la SEC ID NO: 206; o

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido de la SEC ID NO: 206; o

- una secuencia de aminoácido que tiene 2 ó solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido de la SEC ID NO: 206;

y

c) FR3 comprende o es:

- la secuencia de aminoácido de la SEC ID NO: 274; o

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de

identidad de secuencia con la secuencia de aminoácido de la SEC ID NO: 274; o

- una secuencia de aminoácido que tiene solamente 1 diferencia de aminoácido con la secuencia de aminoácido de la SEC ID NO: 274.

y

d) FR4 comprende o es:

- la secuencia de aminoácido de la SEC ID NO: 342; o

- una secuencia de aminoácido que tiene al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido de la SEC ID NO: 342; o

- una secuencia de aminoácido que tiene solamente 1 diferencia de aminoácido con la secuencia de aminoácido de la SEC ID NO: 342;

en las cuales las diferencias de aminoácidos presente en las secuencias de estructura son más preferiblemente como se describen en este documento.

En otro aspecto, la invención se refiere a un Nanocuerpo con una secuencia de aminoácido que es elegida del grupo que consiste de las SEC ID Nos.: 52 hasta 60, del grupo que consiste de las SEC ID Nos.: 76 hasta 86, del grupo que consiste de las SEC ID Nos.: 95 hasta 99, del grupo que consiste de las SEC ID Nos. 105 hasta 129 o del

grupo que consiste a partir de la secuencia de aminoácido que tiene más de 80%, preferiblemente más de 90%, más preferiblemente más de 95%, tal como 99% ó más de "identidad de secuencia" (como se define en este documento) con una ó más de la secuencia de aminoácido de la SEC ID Nos.: 52 hasta 60, SEC ID Nos.: 6 hasta 86, SEC ID Nos.: 95 hasta 99 ó SEC ID Nos. 105 hasta 129, en las cuales, la última secuencia de aminoácido tiene más preferiblemente, secuencias de estructura que son como se definen adicionalmente posteriormente, bajo la descripción general de las secuencias de estructura de Nanocuerpos.

Por consiguiente, en una modalidad específica pero no limitante, las últimas secuencias de aminoácidos son "humanizadas", como se describe además en este documento.

Más preferiblemente, los Nanocuerpos de la invención son elegidos del grupo que consiste de la SEC ID Nos.: 52 hasta 60, del grupo que consiste de las SEC ID Nos.: 76 hasta 86, del grupo que consiste de las SEC ID Nos.: 95 hasta 99, o del grupo que consiste de las SEC ID Nos. 105 hasta 129, de las cuales, los Nanocuerpos "humanizados" de las SEC ID Nos. 76 hasta 86 y SEC ID Nos.: 95 hasta 99 pueden ser particularmente preferidas.

Como se mencionó anteriormente, un Nanocuerpo particularmente preferido de la invención, es el clon

PMP1C2 (TNF1; SEC ID NO: 52). De este modo, en un aspecto preferido, la invención se refiere a un Nanocuerpo con una secuencia de aminoácido que es elegida del grupo que consiste de las SEC ID NO: 52 o del grupo que consiste de la secuencia de aminoácido que tiene más de 80%, preferiblemente más de 90%, más preferiblemente más de 95%, tal como 99% ó más de "identidad de secuencia" (como se define en este documento) con la secuencia de aminoácido de la SEC ID NO: 52, en los cuales la última secuencia de aminoácido más preferiblemente tienen secuencias de estructura que son como se definen además posteriormente, bajo la descripción general de las secuencias de estructura de Nanocuerpos.

Particularmente preferidas, son variantes humanizadas del clon PMP1C2 (TNF1; SEC ID NO: 52). Algunos ejemplos preferidos, pero no limitantes de tales variantes humanizadas son los clones TNF13 (SEC ID NO: 76), TNF14 (SEC ID NO:77), TNF29 (SEC ID NO: 95) y TNF 30 (SEC ID NO: 96). De este modo, en un aspecto preferido, la invención se refiere a un Nanocuerpo humanizado con una secuencia de aminoácido que es elegida del grupo que consiste de las SEC ID Nos.: 76, 77, 95 ó 96, o del grupo que consiste de la secuencia de aminoácido que tiene más de 80%, preferiblemente más de 90%, más preferiblemente más de 95%, tal como 99% ó más de "identidad de secuencia" (como se

define en este documento) con una de las secuencias de aminoácido de las SEC ID Nos.: 76, 77, 95 ó 96, en las cuales la última secuencia de aminoácido más preferiblemente tiene secuencias de estructura que son como se definen además posteriormente, bajo la descripción general de las secuencias de estructura de Nanocuerpos.

De conformidad con una modalidad preferida, el Nanocuerpo de la invención es una variante humanizada del Nanocuerpo TNF 1 (SEC ID NO: 52).

Algunos aspectos preferidos de esta modalidad de la invención son:

XLIX) Una variante humanizada del Nanocuerpo TNF1, el cual tiene una relación Koff para TNF mejor de 2.10^{-3} (1/s), preferiblemente mejor que 1.10^{-3} (1/s).

L) Una variante humanizada del Nanocuerpo TNF1, el cual tiene un valor EC50 en el ensayo a base de célula usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que el valor EC50 del Nanocuerpo VHH 3E (SEC ID NO:4) del documento WO 04/041862 en el mismo ensayo.

LI) Una variante humanizada del Nanocuerpo TNF 1, el cual tiene un valor EC50 en el ensayo a base de célula usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que 5nM.

LII) Una variante humanizada del Nanocuerpo TNF 1, el

cual tiene un valor EC50 en el ensayo a base de célula usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que 3nM.

LIII) Una proteína o polipéptido, el cual comprende o consiste esencialmente de al menos un Nanocuerpo el cual es una variante humanizada del Nanocuerpo TNF 1 de conformidad con cualquiera de XLIX) hasta LII).

LIV) Una proteína o polipéptido, el cual comprende o consiste esencialmente de dos Nanocuerpos los cuales son variantes humanizadas del Nanocuerpo TNF1 de conformidad con cualquiera de XLIX) hasta LII) (opcionalmente enlazadas vía un enlazador).

LV) Una proteína o polipéptido, el cual comprende o consiste esencialmente de dos Nanocuerpos los cuales son variantes humanizadas del Nanocuerpo TNF1 de conformidad con cualquiera de XLIX) hasta LII) (opcionalmente enlazadas vía un enlazador), y el cual es de manera tal que dicha proteína o polipéptido, después del enlace a un trímero de TNF, es capaz de inhibir o reducir la reticulación del receptor de TNF que es mediada por dicho trímero de TNF y/o la transducción de señal que es mediada por tal reticulación del receptor.

LVI) Una proteína o polipéptido, el cual comprende o consiste esencialmente de dos Nanocuerpos los cuales

son variantes humanizadas del Nanocuerpo TNF1 de conformidad con cualquiera de XLIX) hasta LII) y el cual es capaz de enlazarse intramolecularmente con al menos dos sitios de enlace al receptor TNF en un trímero de TNF.

LVII) Una proteína o polipéptido el cual comprende o consiste esencialmente del polipéptido TNF 7 (SEC ID NO: 73), en los cuales ambos Nanocuerpos TNF1 han sido humanizados.

LVIII) Una proteína o polipéptido el cual comprende o consiste esencialmente del polipéptido TNF 7 (SEC ID NO: 73), en los cuales ambos Nanocuerpos TNF1 han sido humanizados, y el cual es pegilado.

LIX) Una proteína o polipéptido, el cual comprende o consiste esencialmente de dos Nanocuerpos los cuales son variantes humanizadas del Nanocuerpo TNF1 de conformidad con cualquiera de XLIX) hasta LII), y el cual además, comprende al menos un Nanocuerpo dirigido contra albúmina de suero humana.

LX) Una proteína o polipéptido, el cual comprende o consiste esencialmente de dos Nanocuerpos los cuales son variantes humanizadas del Nanocuerpo TNF1 de conformidad con cualquiera de XLIX) hasta LII), los cuales son cada uno enlazados (opcionalmente enlazados vía un enlazador) hasta un Nanocuerpo dirigido contra

albúmina de suero humana.

- Una proteína o polipéptido de conformidad con cualquiera de LIII) hasta LX), en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es un Nanocuerpo humanizado.
- Una proteína o polipéptido de conformidad con cualquiera de LIII) hasta LX), en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es una variante humanizada del Nanocuerpo ALB 1 (SEC ID NO: 63).
- Una proteína o polipéptido de conformidad con cualquiera de LIII) hasta LX), en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es uno elegido del grupo que consiste de ALB 3 (SEC ID NO: 87), ALB 4 (SEC ID NO: 88), ALB 5 (SEC ID NO: 89), ALB 6 (SEC ID NO: 100), ALB 7 (SEC ID NO: 101), ALB 8 (SEC ID NO: 102) ALB 9 (SEC ID NO: 103) y ALB 10 (SEC ID NO: 104).
- Una proteína o polipéptido de conformidad con cualquiera de LIII) hasta LX), en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es ALB 8.
- Una proteína o polipéptido de conformidad con cualquiera de LIII) hasta LX), el cual comprende o consiste esencialmente del polipéptido TNF24 (SEC ID

NO: 90), en los cuales tanto el Nanocuerpo TNF1 así como también el Nanocuerpo ALB TNF1 han sido humanizados.

- Una proteína o polipéptido de conformidad con cualquiera de LIII) hasta LX), el cual comprende o consiste esencialmente de dos Nanocuerpos TNF 30 y un Nanocuerpo ALB 8.

De conformidad con una modalidad preferida, el Nanocuerpo de la invención es una variante humanizada del Nanocuerpo TNF 3 (SEC ID NO:60).

Algunos aspectos preferidos de esta modalidad de la invención son:

LXI) Una variante humanizada del Nanocuerpo TNF 3, el cual tiene una relación Koff para TNF mejor de 2.10-3 (1/s), preferiblemente mejor que 1.10-3 (1/s).

LXII) Una variante humanizada del Nanocuerpo TNF 3, el cual tiene un valor EC50 en el ensayo a base de célula usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que el valor EC50 del Nanocuerpo VHH 3E (SEC ID NO:4) del documento WO 04/041862 en el mismo ensayo.

LXIII) Una variante humanizada del Nanocuerpo TNF 3, el cual tiene un valor EC50 en el ensayo a base de célula usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que

5nM.

LXIV) Una variante humanizada del Nanocuerpo TNF 3, el cual tiene un valor EC50 en el ensayo a base de célula usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que 3nM.

LXV) Una proteína o polipéptido, el cual comprende o consiste esencialmente de al menos un Nanocuerpo el cual es una variante humanizada del Nanocuerpo TNF 3 de conformidad con cualquiera de LXI) hasta LXIV).

LXVI) Una proteína o polipéptido, el cual comprende o consiste esencialmente de dos Nanocuerpos los cuales son variantes humanizadas del Nanocuerpo TNF 3 de conformidad con cualquiera de LXI) hasta LXIV) (opcionalmente enlazadas vía un enlazador).

LXVII) Una proteína o polipéptido, el cual comprende o consiste esencialmente de dos Nanocuerpos los cuales son variantes humanizadas del Nanocuerpo TNF 3 de conformidad con cualquiera de LXI) hasta LXIV) (opcionalmente enlazadas vía un enlazador), y el cual es de manera tal que dicha proteína o polipéptido, después del enlace a un trímero de TNF, es capaz de inhibir o reducir la reticulación del receptor de TNF que es mediada por dicho trímero de TNF y/o la transducción de señal que es mediada por tal

reticulación del receptor.

LXVIII) Una proteína o polipéptido, el cual comprende o consiste esencialmente de dos Nanocuerpos los cuales son variantes humanizadas del Nanocuerpo TNF 3 de conformidad con cualquiera de LXI) hasta LXIV) y el cual es capaz de enlazarse intramolecularmente con al menos dos sitios de enlace al receptor TNF en un trímero de TNF.

LXIX) Una proteína o polipéptido el cual comprende o consiste esencialmente del polipéptido TNF 6 (SEC ID NO: 72) ó TNF 9 (SEC ID NO: 75), en los cuales ambos Nanocuerpos TNF 3 han sido humanizados.

LXX) Una proteína o polipéptido el cual comprende o consiste esencialmente del polipéptido TNF 6 (SEC ID NO: 72) ó TNF 9 (SEC ID NO: 75), en los cuales ambos Nanocuerpos TNF 3 han sido humanizados, y el cual es pegilado.

LXXI) Una proteína o polipéptido, el cual comprende o consiste esencialmente de dos Nanocuerpos los cuales son variantes humanizadas del Nanocuerpo TNF 3 de conformidad con cualquiera de LXI) hasta LXIV), y el cual además, comprende al menos un Nanocuerpo dirigido contra albúmina de suero humana.

LXXII) Una proteína o polipéptido, el cual comprende o consiste esencialmente de dos Nanocuerpos los cuales

son variantes humanizadas del Nanocuerpo TNF 3 de conformidad con cualquiera de LXI) hasta LXIV), las cuales son cada una enlazadas (opcionalmente enlazadas vía un enlazador) hasta un Nanocuerpo dirigido contra albúmina de suero humana.

- Una proteína o polipéptido de conformidad con cualquiera de LXV) hasta LXXII), en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es un Nanocuerpo humanizado.
- Una proteína o polipéptido de conformidad con cualquiera de LXV) hasta LXXII), en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es una variante humanizada del Nanocuerpo ALB 1 (SEC ID NO: 63).
- Una proteína o polipéptido de conformidad con cualquiera de LXV) hasta LXXII), en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero humana es uno elegido del grupo que consiste de ALB 3 (SEC ID NO: 87), ALB 4 (SEC ID NO: 88), ALB 5 (SEC ID NO: 89), ALB 6 (SEC ID NO: 100), ALB 7 (SEC ID NO: 101), ALB 8 (SEC ID NO: 102) ALB 9 (SEC ID NO: 103) y ALB 10 (SEC ID NO: 104).
- Una proteína o polipéptido de conformidad con cualquiera de LXV) hasta LXXII), en los cuales al menos un Nanocuerpo dirigido contra albúmina de suero

humana es ALB 8.

- Una proteína o polipéptido de conformidad con cualquiera de LXV) hasta LXXII), el cual comprende o consiste esencialmente del polipéptido TNF26 (SEC ID NO: 92), en los cuales tanto los Nanocuerpos TNF 3 así como también el Nanocuerpo ALB 1 han sido humanizados.

En otro aspecto, la invención se refiere a un polipéptido que comprende o consiste esencialmente de al menos, un Nanocuerpo contra TNF-alfa como se define en este documento. Tales polipéptidos también son referidos en este documento como "polipéptidos de la invención", y pueden ser como se describe además aquí posteriormente y/o como se describe en general, en el documento WO 04/041862 para los Nanocuerpos descritos aquí, y pueden por ejemplo, ser polipéptidos multivalente o polipéptidos multiespecíficos, nuevamente como se describe además aquí posteriormente.

Preferiblemente, un polipéptido de la invención es ya sea bivalente o trivalente (es decir, que comprende dos o tres Nanocuerpos de la invención, respectivamente, opcionalmente ligados vía uno o dos enlazadores como se define posteriormente, respectivamente), o un polipéptido multiespecífico, que comprende uno o dos, y preferiblemente dos, Nanocuerpos de la invención y al menos, un Nanocuerpo dirigido contra una proteína del suero, y en particular, contra una proteína del suero humano, tal como contra

albúmina de suero humana.

En modalidades preferidas pero no limitantes, los Nanocuerpos de la invención presentes en los polipéptidos de la invención, son elegidos del grupo que consiste de las SEC ID NOs: 52 a 60 y SEC ID NOs. 105-129 o de las variantes humanizadas de los mismos, y en particular, de los Nanocuerpos "humanizados" de las SEC ID NOS: 76 a 86 y SEC ID NOS:95 a 99. Los Nanocuerpos contra albúmina de suero humana presente en los polipéptidos de la invención, son preferiblemente como se definen posteriormente, y son más preferiblemente elegidas del grupo que consiste de las SEC ID NOS: 61 a 67, SEC ID NOS: 87 a 89 y SEC ID NOS: 100-104, y en particular, de los Nanocuerpos "humanizados" contra albúmina de suero humano de la SEC ID NOS. 76 a 86 y SEC ID NOS: 100-104.

Con respecto a los Nanocuerpos que están presentes en los polipéptidos de la invención, será claro para la persona experta, que los Nanocuerpos que son mencionados en este documento como "preferidos" (o como "más preferidos", "aún más preferidos", etc.), son también preferidos (o más preferidos, o aún más preferidos, etc.), para uso en los polipéptidos descritos en este documento. De este modo, los polipéptidos que comprenden o consisten esencialmente de uno o más Nanocuerpos "preferidos" de la invención, en general, serán preferidos, y los polipéptidos

que comprenden o consisten esencialmente de uno o más Nanocuerpos "más preferidos" de la invención, en general, serán más preferidos, etc.

De este modo, en la invención, los polipéptidos que comprenden uno o más Nanocuerpos que consisten esencialmente de una de las variantes preferidas del clon PMP1C2 (TNF1; SEC ID NO;52), en el cual dichas variantes preferidas son como se definen en este documento, son particularmente preferidas. Aún más preferido, son polipéptidos que comprenden uno o más Nanocuerpos que esencialmente consisten de las variantes humanizadas del clon PMP1C2 (TNF1; SEC ID NO:52), en el cual, dichas variantes humanizadas son como se definen en este documento (los ejemplos son, sin limitación, TNF13, TNF14, TNF29 y TNF30). El TNF30 es un "bloque de construcción" humanizado particularmente preferido para uso en los polipéptidos de la invención.

Algunos ejemplos preferidos, pero no limitantes de tales proteínas y polipéptidos son el PMP1C2 mismo, las variantes humanizadas TNF13, TNF14, TN29 y TNF30; los constructos de la SEC ID NO:70 (TNF4), SEC ID NO:73 (TNF7), SEC ID NO:90 (TNF24), SEC ID NO:93 (TNF27); y los constructos de la SEC ID NO:417 (TNF60), SEC ID NO:419 (TNF55) y SEC ID NO:420 (TNF56), en los cuales, los tres últimos constructos contienen la variante humanizada TNF30

como un bloque de construcción.

Como se mencionó en este documento, los Nanocuerpos y constructos descritos en este documento, pueden ser pegilados, o contener uno o más residuos de aminoácido (adicionales), que permiten la pegilación y/o facilitan la pegilación. Dos ejemplos preferidos, pero no limitantes de tales polipéptidos son, TNF55 y TNF56, los cuales ambos contienen un residuo de cisteína adicional para la unión fácil de un grupo PEG.

Algunos ejemplos preferidos, pero no limitantes de polipéptidos de la invención, son los polipéptidos bivalentes de la invención de las SEC ID NOS: 70 a 75 y los polipéptidos multiespecíficos de la invención de las SEC ID NO:90 hasta 94, y las SEC ID NOS 417 hasta 420.

Como se puede ver a partir de los datos presentados abajo, y en particular de los datos dados en el Ejemplo Comparativo, los Nanocuerpos y/o polipéptidos de la invención, tienen propiedades mejoradas. En particular, las proteínas y polipéptidos de la invención pueden tener afinidad mejorada para TNF-alfa humano (expresado como el valor EC_{50} en el ensayo KYM descrito en este documento), comparados con los biológicos anti-TNF comercialmente disponibles Enbrel™, Humira™, y Remicade™. También, los Nanocuerpos descritos en este documento, pueden tener una afinidad mejorada para TNF-alfa comparada con el mejor

desempeño del Nanocuerpo descrito en la solicitud Internacional WO 04/041862. Puede esperarse además, que los polipéptidos de la invención que comprenden al menos, uno de los Nanocuerpos de la invención, también tendrán propiedades mejoradas comparadas con los polipéptidos que comprenden solamente los Nanocuerpos contra TNF-alfa descritos en el documento WO 04/041862.

Más en particular, un polipéptido como se describe en este documento, que comprenden dos o más (y preferiblemente dos) Nanocuerpos como en este documento (y opcionalmente por ejemplo, un Nanocuerpo contra albúmina de suero humana), que tiene un valor EC50 en el ensayo a base de la célula usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que el valor de EC50 de Humira® en Remicade®, y preferiblemente, también mejor que Enbrel® en el mismo ensayo.

Por ejemplo, tal proteína o polipéptido, preferiblemente tiene un valor EC50 en el ensayo a base de células usando células KYM descritas en el Ejemplo 1, bajo 3) del documento WO 04/041862 que es mejor de 0.2 nM, preferiblemente, mejor que 0.1 nM, tal como mejor que 0.7 nM y en particular, mejor que 0.4 nM.

También se ha mostrado por los solicitantes, que los Nanocuerpos contra TNF-alfa de ratón y polipéptidos que comprenden Nanocuerpos contra TNF-alfa de ratón, muestran

una actividad biológica benéfica en los siguientes modelos de enfermedad (datos no mostrados):

- El modelo de dextran sulfato de sodio ("DSS") de colitis, usando tanto ratones regulares como también ratones agénicos IL-10, como se describe por Okayasu et al. (Gastroenterol 1990, 98(3): 694).
- El modelo de artritis inducida por colágeno ("CIA") de artritis ("RA"), como se describe por Courtenay et al. (Nature 1980, 283(5748): 666), usando tanto ratones regulares como también ratones agénicos IL-10;
- El modelo de ratón agénico IL-10 de IBD, como por ejemplo, se describe por Rennick et al. (Clin Immunol Immunopathol 1995, 76(3 Pt 2): S174).
- El modelo Kollias de RA como por se describe por ejemplo, por Keffer et al. (EMBO J 1991, 10(13): 4025).
- El modelo de ácido 2,4,6-trinitrobencensulfónico ("TNBS") de IBD, como se describe por Elson *et al.*, (J Immunol 1996, 157(5): 2174).
- El modelo CIA de RA, descrito por Koppieters et al (manuscrito en preparación);
- El modelo de fibroblasto derivado del sinovial (descrito posteriormente); y
- El modelo de bolsa de aire murino.

Preferiblemente, los Nanocuerpos descritos en

este documento, son mejor que el Nanocuerpo 1A a partir del documento WO 04/041862 en al menos, uno de estos modelos, y preferiblemente en todos estos modelos; y más preferiblemente, mejor que el Nanocuerpo 3E del documento WO 04/041862 en al menos, uno de estos modelos, y preferiblemente, en todos estos modelos. También, los polipéptidos descritos en este documento, son preferiblemente equivalentes a, o mejores que Humira® o Remicade® en al menos, uno de estos modelos, y preferiblemente, en todos estos modelos; y más preferiblemente, también equivalentes a, o mejores que Enbrel® en al menos, uno de estos modelos, y preferiblemente en todos estos modelos.

Estos datos confirman que los Nanocuerpos contra TNF-alfa y polipéptidos que contienen a los mismos, tales como los Nanocuerpos y polipéptidos descritos en el documento WO 04/041862 y en particular, los Nanocuerpos y polipéptidos descritos en este documento, deben tener eficacia terapéutica contra enfermedades y trastornos mediados por TNF, tal como las enfermedades y trastornos mencionados anteriormente.

En otro aspecto, la invención se refiere a un ácido nucleico que codifica un Nanocuerpo de la invención y/o un polipéptido de la invención. Tal ácido nucleico también se referirá abajo como un "ácido nucleico de la

invención", y puede por ejemplo, estar en la forma de un constructo genético, como se define posteriormente.

En otro aspecto, la invención se refiere a un hospedero o célula hospedera que expresa o es capaz de expresar un Nanocuerpo de la invención y/o un polipéptido de la invención; y/o que contiene un ácido nucleico que codifica un Nanocuerpo de la invención y/o un polipéptido de la invención. Tal hospedero o célula hospedera, puede también ser análoga al hospedero o células hospederas descritas en el documento WO 04/041862, pero que expresan o son capaz de expresar un Nanocuerpo de la invención y/o un polipéptido de la invención y/o que contiene un ácido nucleico como se describe en este documento.

La invención además se refiere a un producto o composición que contiene o comprende un Nanocuerpo de la invención, un polipéptido de la invención; y/o un ácido nucleico de la invención. Tal producto o composición puede por ejemplo, ser una composición farmacéutica (como se describe abajo), o un producto o composición para uso de diagnóstico (como también se describe abajo). Tal producto o composición puede también ser análoga a los productos y composiciones descritas en el documento WO 04/041862, pero que contienen o comprenden un Nanocuerpo de la invención, un polipéptido de la invención o un ácido nucleico de la invención.

La invención además se refiere a métodos para

preparar o generar los Nanocuerpos, polipéptidos, ácidos nucleicos, células hospederas, productos y composiciones como se describe en este documento, en el cual, los métodos son como se describen además posteriormente. También, en general, los Nanocuerpos, polipéptidos, ácidos nucleicos, células hospederas, productos y composiciones descritas en este documento, también pueden ser preparados y usados en una manera análoga a la manera descrita en el documento WO 04/041862.

La invención además se refiere a aplicaciones y usos de los Nanocuerpos anteriores, polipéptidos, ácidos nucleicos, células hospederas, productos y composiciones descritas en este documento, en la cual las aplicaciones y usos incluyen, pero no se limitan a, las aplicaciones y usos descritos aquí posteriormente y/o los usos adicionales y aplicaciones para Nanocuerpos contra TNF-alfa y/o para polipéptidos que contienen los mismos en el documento WO 04/041862.

Otros aspectos, modalidades, ventajas y solicitudes de la invención, llegarán a ser claros a partir de la descripción adicional aquí posteriormente.

DESCRIPCIÓN DETALLADA DE LA INVENCIÓN

Los aspectos anteriores y otros y modalidades de la invención, llegarán a ser más claros a partir de la

descripción adicional aquí posteriormente, en la cual:

a) A menos que se indique o se defina de otro modo, todos los términos usados tienen su significado usual en la técnica, la cual será clara para la persona experta. La referencia es por ejemplo, hecha a los manuales estándares, tales como, Sambrook et al, "Molecular Cloning: A Laboratory Manual" (2da.Ed.), Vols. 1-3, Cold Spring Harbor Laboratory Press (1989); F. Ausubel et al, eds., "Current protocols in molecular biology", Green Publishing and Wiley Interscience, New York (1987); Lewin, "Genes II", John Wiley & Sons, New York, N.Y., (1985); Old et al., "Principles of Gene Manipulation: An Introduction to Genetic Engineering", 2da. edición, University of California Press, Berkeley, CA (1981); Roitt et al., "Immunology" (6th. Ed.), Mosby/Elsevier, Edinburgh (2001); Roitt et al., Roitt's Essential Immunology, 10th Ed Blackwell Publishing, UK (2001), y Janeway et al, "Immunobiology" (6th Ed.), Garland Science Publishing/Churchill Livingstone, New York (2005), así como también, a la técnica antecedente general mencionada en este documento;

b) A menos que se indique de otro modo, el término "secuencia de inmunoglobulina" - si se usa en este documento para referirse a un anticuerpo de cadena pesada o a un anticuerpo de 4 cadenas convencional- se usa como un término

general para incluir tanto el anticuerpo de tamaño completo, las cadenas individuales de los mismos, así como también todas las partes, dominios o fragmentos de los mismos (que incluyen, pero no se limitan a, dominios o fragmentos de enlace al antígeno, tales como dominios V_{HH} o dominios V_H/V_L, respectivamente. Además, el término "secuencia" como se usa en este documento (por ejemplo, en términos como "secuencia de inmunoglobulina", "secuencia de anticuerpo", "secuencia de dominio variable", "secuencia V_{HH}" o "secuencia de proteína"), deben ser en general, entendidos por incluir tanto la secuencia de aminoácido relevante, así como también secuencias de ácido nucleico o secuencias de nucleótido que codifican a los mismos, a menos que el contexto requiera una interpretación más limitada.

c) A menos que se indique de otro modo, todos los motos, etapas, técnicas y manipulaciones que no son específicamente descritas en detalle, pueden ser realizado y han sido realizados en una manera conocida per se, como será claro para las personas expertas. La referencia es hecha nuevamente, por ejemplo, a los manuales estándares, a la técnica de antecedente general referida anteriormente y a las referencias adicionales citadas en este documento;

d) Los residuos de aminoácido serán indicados de conformidad con el código de aminoácido de una letra o tres letras, como se menciona en la Tabla 1;

Tabla 1: Código de aminoácido de una letra y tres letras

No polar, no cargado (a pH 6.0 – 7.0)[3]	Alanina	Ala	A
	Valina	Val	V
	Leucina	Leu	L
	Isoleucina	Ile	I
	Fenilalanina	Phe	F
	Metionina[1]	Met	M
	Triptofano	Trp	W
	Prolina	Pro	P
Polar, no cargado (a pH 6.0-7.0)	Glicina[2]	Gly	G
	Serina	Ser	S
	Treonina	Thr	T
	Cisteína	Cys	C
	Asparagina	Asn	N
	Glutamina	Gln	Q
	Tirosina	Tyr	Y
Polar, cargado (a pH 6.0-7.0)	Lisina	Lys	K
	Arginina	Arg	R
	Histidina[4]	His	H
	Aspartato	Asp	D
	Glutamato	Glu	E

Notas:

(1) Algunas veces también considerado por ser un aminoácido polar, no cargado.

(2) Algunas veces también considerado por ser un aminoácido no polar, no cargado.

(3) Como será claro para las personas expertas, el hecho de que un residuo de aminoácido es referido en esta Tabla por ser ya sea cargado o no cargado a pH 6.0 hasta 7.0, no se refleja en alguna forma, en la carga que dicho residuo de aminoácido puede tener a un pH inferior que 6.0 y/o a un pH superior que 7.0; los residuos de aminoácido mencionados en la Tabla 5, pueden ser ya sea cargados y/o no cargados, a tal pH superior o inferior, como será claro para las personas expertas.

(4) Como se sabe en la técnica, la carga de un residuo His es mayormente dependiente de aún cambios menores en pH, pero un residuo His puede en general, ser considerado esencialmente no cargado a un pH de aproximadamente 6.5.

837

e) Para propósitos de comparar dos o más secuencias de nucleótidos, el porcentaje de "identidad de secuencia" entre una primera secuencia de nucleótido y una segunda secuencia de nucleótido, puede ser calculada dividiendo [el número de nucleótidos de la primera secuencia de nucleótidos que son idénticos a los nucleótidos en las posiciones correspondientes en la segunda secuencia de nucleótido] por [el número total de nucleótidos en la primera secuencia de nucleótido], y multiplicando por [100%], en tal caso, supresión, inserción, sustitución o adición de un nucleótido en la segunda secuencia de nucleótido -comparada con la primera secuencia de nucleótido-, es considerado como una diferencia en un nucleótido único (posición).

Alternativamente, el grado de identidad de secuencia entre dos o más secuencias de nucleótidos puede ser calculado usando un algoritmo de ordenador conocido para alineación de secuencia, tal como NCBI Blast v2.0, usando ajustes estándares.

Algunas otras técnicas, algoritmos de ordenador y ajustes para determinar el grado de identidad de secuencia son por ejemplo, descritos en los documentos WO 04/037999, EP 0 967 284, EP 1 085 089, WO 00/55318, WO 00/78972, WO 98/49185 y GB 2 357 768-A.

Usualmente, para propósitos de determinar el

838

porcentaje de "identidad de secuencia" entre dos secuencias de nucleótido de conformidad con el método de cálculo resumido aquí anteriormente, la secuencia de nucleótido con el número más grande de nucleótidos, se tomará como la "primera" secuencia de nucleótidos, y la otra secuencia de nucleótidos será tomada como la "segunda" secuencia de nucleótidos.

f) Para propósitos de comparación de dos o más secuencias de aminoácido, el porcentaje de "identidad de secuencia" entre una primera secuencia de aminoácido y una secuencia de aminoácido, puede ser calculado dividiendo [el número de residuos de aminoácido en la primera secuencia de aminoácido, que son idénticos a los residuos de aminoácido en las posiciones correspondientes en la segunda secuencia de aminoácido] por [el número total de nucleótidos en la primera secuencia de aminoácido] y multiplicando por [100%], en los cuales, cada supresión, inserción, substitución o adición de un residuo de aminoácido en la segunda secuencia de aminoácido -comparada con la primera secuencia de aminoácido- es considerada como la diferencia en un residuo de aminoácido único (posición), es decir, como una "diferencia de aminoácido", como se define abajo.

Alternativamente, el grado de identidad de secuencia entre dos secuencias de aminoácido, puede ser calculado usando un algoritmo de ordenador conocido, tal

como aquellos mencionados anteriormente para determinar el grado de identidad de secuencia para secuencias de nucleótidos, nuevamente usando ajustes estándares.

Usualmente, para propósitos de terminar el porcentaje de "identidad de secuencia", entre dos secuencias de aminoácido de conformidad con el método de cálculo resumido aquí anteriormente, la secuencia de aminoácido con el número más grande de residuos de aminoácido, será tomada como la "primera" secuencia de aminoácido, y la otra secuencia de aminoácido será tomada como la "segunda" secuencia de aminoácido.

También, determinando el grado de identidad de secuencia entre las dos secuencias de aminoácido, la persona experta puede tomar en cuenta las así llamadas sustituciones de aminoácido "conservativas", las cuales pueden en general, ser descritas como sustituciones de aminoácido en las cuales, un residuo de aminoácido es reemplazado con otro residuo de aminoácido de estructura química similar y el cual tiene poca o ninguna influencia en la función, actividad u otras propiedades biológicas del polipéptido. Tales sustituciones de aminoácido conservativas son bien conocidas en la técnica, por ejemplo, a partir de los documentos WO 04/037999, GB-A-2 357 768, WO 98/49185, WO 00/46383 y WO 01/09300; y tipos (preferidos) y/o combinaciones de tales sustituciones,

840

pueden ser seleccionadas en base a las enseñanzas pertinentes a partir del documento WO 04/037999, así como también el documento WO 98/49185 y a partir de las referencias adicionales citadas en este documento.

Tales sustituciones conservativas preferiblemente son sustituciones en las cuales, un aminoácido dentro de los siguientes grupos (a) - (e) es sustituido por otro residuo de aminoácido dentro del mismo grupo: (a) residuos alifáticos menores, no polares o ligeramente polares: Ala, Ser, Thr, Pro y Gly; (b) residuos polares, negativamente cargados y sus amidas (no cargadas): Asp, Asn, Glu y Gln; (c) residuos polares, positivamente cargados: His, Arg y Lys; (d) residuos alifáticos grandes, no polares: Met, Leu, He, Val y Cys; y (e) residuos aromáticos: Phe, Tyr y Trp.

Sustituciones conservativas particularmente preferidas son como sigue: Ala en Gly o es Ser; Arg en Lys; Asn en Gln o en His; Asp en Glu; Cys en Ser; Gln en Asn; Glu en Asp; Gly en Ala o en Pro; His en Asn o en Gln; He en Leu o en Val; Leu en He o en Val; Lys en Arg, en Gln o en Glu; Met en Leu, en Tyr o en He; Phe en Met, en Leu o en Tyr; Ser en Thr; Thr en Ser; Trp en Tyr; Tyr en Trp; y/o Phe en Val, en He o en Leu.

Cualquiera de las sustituciones de aminoácido aplicadas a los polipéptidos descritos en este documento, pueden también basarse en el análisis de las frecuencias de

PCT

variaciones de aminoácidos entre proteínas homólogas de diferentes especies desarrolladas por Schulz et al., Principles of Protein Structure, Springer-Verlag, 1978, en los análisis de los potenciales que forman estructura desarrollados por Chou y Fasman, Biochemistry 13:1211, 1974 y Adv. Enzymol., 47:45-149, 1978, y en los análisis de patrones de hidrofobicidad en proteínas desarrolladas por Eisenberg et al., Proc. Nad. Acad Sci. USA 81: 140-144, 1984; Kyte & Doolittle; J Molec. Biol. 157: 105-132, 198 1, and Goldman et al., Ann. Rev. Biophys. Chem. 15: 321-353, 1986, todas incorporadas en este documento en su totalidad por referencia. La información en la estructura primaria, secundaria y terciaria de Nanocuerpos dada en la descripción en este documento y en la técnica de antecedente general citada anteriormente. También, para este propósito, la estructura cristalina de un dominio VHH de una llama es por ejemplo, dado por Desmyter et al., Nature Structural Biology, Vol. 3, 9, 803 (1996); Spinelli et al., Natural Structural Biology (1996); 3, 752-757; and Decanniere et al., Structure, Vol. 7, 4, 361 (1999). Información adicional acerca de los residuos de aminoácido que en los dominios VH convencionales forman la interfaz de VH/VL y las sustituciones camelizantes potenciales en estas posiciones, se pueden encontrar en la técnica anterior en los Nanocuerpos citados en este documento;

842

g) secuencias de aminoácidos y secuencias de ácidos nucleicos se dice son "exactamente las mismas", si tienen 100% de identidad de secuencia (como se define en este documento), en su longitud completa;

h) cuando se comparan dos secuencias de aminoácido, el término "diferencia de aminoácido", se refiere a una inserción, supresión o sustitución de un residuo de aminoácido único en una posición de la primera secuencia, comparada con la segunda secuencia; se entiende que dos secuencias de aminoácido pueden contener una, dos o más de tales diferencias de aminoácidos;

i) una secuencia de aminoácido o una secuencia de aminoácido es considerada por estar "(en) (forma) esencialmente aislada"-, por ejemplo, comparada con su fuente biológica nativa y/o el medio de reacción o medio de cultivo a partir del cual se ha obtenido -, cuando se ha separado de al menos, otro componente con el cual está usualmente asociado en dicha fuente o medio, tal como otro ácido nucleico, otra proteína/polipéptido, otro componente biológico o macromolécula o al menos, un contaminante, impureza o componente menor. En particular, una secuencia de ácido nucleico o secuencia de aminoácido se considera "esencialmente aislada", cuando se ha purificada en al menos, 2 veces, en particular, al menos 10 veces, más en particular, al menos 100 veces, y hasta 1000 veces o más.

Una secuencia de ácido nucleico o secuencia de aminoácido que está en "forma esencialmente aislada", es preferiblemente esencialmente homogénea, como se determina usando una técnica adecuada, tal como una técnica cromatográfica adecuada, tal como poliacrilamida-electroforesis;

j) El término "dominio" como se usa en este documento, se refiere a una región globular de una cadena de anticuerpo, y en particular, a una región globular de un anticuerpo de cadena pesada, o a un polipéptido que consiste esencialmente de tal región globular. Usualmente, tal dominio comprenderá bucles peptídicos (por ejemplo, 3 ó 4 bucles peptídicos) estabilizados, por ejemplo, como una lámina o por enlaces disulfuro.

k) El término "determinante antigénica" se refiere al epítope en el antígeno reconocido por la molécula de enlace al antígeno (tal como un Nanocuerpo o un polipéptido de la invención) y más en particular, por el sitio de enlace al antígeno de dicha molécula. Los términos "determinante antigénica" y "epítope" puede también ser usado intercambiablemente en este documento.

l) Una secuencia de aminoácido (tal como un Nanocuerpo, un anticuerpo, un polipéptido de la invención, o en general una proteína de enlace al antígeno o polipéptido o fragmento del mismo), que pueden enlazarse a,

que tiene afinidad para y/o, tiene especificidad para un determinante antigénico específico, epítope, antígeno o proteína (o por al menos, una parte, fragmento, epítope del mismo), se dice es "contra" o "se dirige contra", dicho determinante antigénico, epítope, antígeno o proteína.

m) El término "especificidad", se refiere al número de diferentes tipos de antígenos o determinantes antigénicas en las cuales una molécula de enlace al antígeno particular o molécula de proteína de enlace al antígeno se puede enlazar (tal como un Nanocuerpo o un polipéptido de la invención). La especificidad de una proteína de enlace al antígeno se puede determinar basada en la afinidad y/o avidez. La afinidad, representada por la constante de equilibrio para la disociación de un antígeno con una proteína de enlace al antígeno (KD), es una medida para la intensidad de enlace entre una determinante antigénica y un sitio de enlace al antígeno en la proteína de enlace al antígeno: a menor valor de KD, más fuerte la resistencia de enlace entre una determinante antigénica y la molécula de enlace al antígeno (alternativamente, la afinidad puede también ser expresada como la constante de afinidad (KA), la cual es 1/KD). Como será claro para las personas expertas (por ejemplo, en base a la descripción adicional en este documento), la afinidad puede ser determinada en una manera conocida por se, dependiendo del

antígeno específico de interés. La avidez es la medida de la resistencia de enlace entre una molécula de enlace al antígeno (tal como un Nanocuerpo o polipéptido de la invención) y el antígeno pertinente. La avidez está relacionada con tanto la afinidad entre una determinante antigénica y su sitio de enlace al antígeno en la molécula de enlace al antígeno y el número de sitios de enlace pertinentes presentes en la molécula de enlace al antígeno. Típicamente, las proteínas de enlace al antígeno (tales como los Nanocuerpos y/o polipéptidos de la invención), se enlazarán con una constante de disociación (KD) de 10^{-5} hasta 10^{-12} moles/litro (M) o menos, y preferiblemente 10^{-7} hasta 10^{-12} moles/litro (M) o menos y más preferiblemente 10^{-8} hasta 10^{-12} moles/litro, y/o con una constante de asociación (K_A) de al menos 10^{-7} M^{-1}, preferiblemente, al menos 10^{8} M^{-1}, más preferiblemente al menos 10^{9} M^{-1}, tal como al menos 10^{12} M^{-1}.

Cualquier valor K_D mayor que 10^{-4} M generalmente se considera que indica enlace no específico. Preferiblemente, un Nanocuerpo o polipéptido de la invención enlazará el antígeno deseado con una K_D menor que 500 nM, preferiblemente menos de 200 nM, más preferiblemente menos de 10 nM, tal como menos de 500 nM. El enlace específico de una proteína de enlace de antígeno a un determinante antígeno o antigénico se puede determinar

en cualquier manera conocida per se, incluyendo, por ejemplo, análisis de Scatchard y/o ensayos de enlace competitivos, tales como radioinmunoensayos (RIA), inmunoensayos de enzima (EIA) y ensayos de competición intercalada, y las diferentes variantes de los mismos conocidas per se en la técnica.

n) como se describe adicionalmente posteriormente, la secuencia de aminoácido y estructura de un Nanocuerpo se puede considerar - sin embargo sin limitarse a esto - que están comprendidas de cuatro regiones de estructura o "FR's", las cuales son referidas en la técnica y posteriormente como *"región de estructura 1"* o *"FR1"*, como *"región de estructura 2"* o *"FR2"*; como *"región de estructura 3"* o *"FR3"*, y como *"región de estructura 4"* o *"FR4"*, respectivamente; las regiones de estructura son interrumpidas por las regiones determinantes complementarias o "CDR's", las cuales son referidas en la técnica como *"región determinante complementaria 1"* o *"CDR1"*; como *"región determinante complementaria 2"* o *"CDR2"*; y como *"región determinante complementaria 3"* o *"CDR3"*, respectivamente;

o) también como se describe adicionalmente posteriormente, el número total de residuos aminoácido en un Nanocuerpo puede estar en la región de 110-120, preferiblemente es 112-115, y muy preferiblemente 113. Sin

embargo, se deberá señalar que las partes, fragmentos o análogos (como se describe adicionalmente posteriormente) de un Nanocuerpo no se limitan particularmente en cuanto a su longitud y/o tamaño, siempre que tales partes, fragmentos o análogos cumplan los requerimientos adicionales resumidos posteriormente y también preferiblemente son adecuados para los propósitos descritos en la presente;

p) los residuos aminoácido de un Nanocuerpo son numerados de conformidad con la numeración general para dominios V_H dados por Kabat et al. ("*Sequence of proteins of immunological interest*", US Public Health Services, NIH Bethesda, MD, Publication No. 91), como se aplica a dominios V_{HH} de Camélidos en el artículo de Riechmann and Muyldermans, referido como anteriormente (véase, por ejemplo figura 2 de la referencia). De conformidad con esta numeración, la FR1 de un Nanocuerpo comprende los residuos aminoácido en las posiciones 1-30, la CDR1 de un Nanocuerpo comprende los residuos aminoácido en las posiciones 31-36, la FR2 de un Nanocuerpo comprende los aminoácidos en las posiciones 36-49, la CDR2 de un Nanocuerpo comprende los residuos aminoácido en las posiciones 50-65, la FR3 de un Nanocuerpo comprende los residuos aminoácido en las posiciones 66-94, la CDR3 de un Nanocuerpo comprende los residuos aminoácido en las posiciones 95-102, y la FR4 de

un Nanocuerpo comprende los residuos aminoácido en la posiciones 103-113. [A este respecto, se deberá señalar que - como también se conoce en la técnica para dominios V_H y para dominios V_{HH} - el número total de residuos aminoácido en cada una de las CDR's puede variar y puede no corresponder al número total de residuos aminoácido indicados por la numeración Kabat (es decir, una o más posiciones de conformidad con la numeración Kabat pueden no ser ocupadas en la secuencia actual, o la secuencia actual puede contener más residuos aminoácido que el número permitido por la numeración Kabat). Esto significa que, generalmente, la numeración de conformidad con Kabat puede o no puede corresponder con la numeración actual de los residuos aminoácido en la secuencia actual. Generalmente, sin embargo, se puede decir que, de conformidad con la numeración de Kabat y sin consideración del número de residuos aminoácido en las CDR's, la posición 1 de conformidad con la numeración Kabat corresponde al inicio de FR1 y viceversa, la posición 36 de conformidad con la numeración Kabat corresponde al inicio de FR2 y viceversa, la posición 66 de conformidad con la numeración Kabat corresponde al inicio de FR3 y viceversa, y la posición 103 de conformidad con la numeración Kabat corresponde al inicio de FR4 y viceversa].

Los métodos alternativos para numerar los

residuos aminoácido de dominios V_H, los métodos también se pueden aplicar de una manera análoga a los dominios V_{HH} de Camélidos y a Nanocuerpos, son el método descrito por Chothia et al. (*Nature* 342, 877-883 (1989)), la así llamada "*definición AbM*" y la así llamada "*definición de contacto*". Sin embargo, en la presente descripción, reivindicaciones y figuras, la numeración de conformidad con Kabat como se aplica a los dominios V_{HH} de Riechmann and Muyldermans será seguida, a menos que se indique de otra forma; y

q) Las figuras, listado de secuencia y la Parte Experimental/Ejemplos solamente se dan para ilustrar adicionalmente la invención y no se deberá interpretar o construir como limitante del alcance de la invención y/o de las reivindicaciones anexas en alguna forma, a menos que se indique explícitamente de otra forma en la presente.

Para una descripción general de anticuerpos de cadena pesada y los dominios variables de los mismos, se hace referencia inter alia a las siguientes referencias, las cuales se mencionan como técnica antecedente general: WO 94/04678, WO 95/04079 y WO 96/34103 de la Vrije Universiteit Brussel; WO 94/25591, WO 99/37681, WO 00/40968, WO 00/43507, WO 00/65057, WO 01/40310, WO 01/44301, EP 1134231 y WO 02/48193 de Unilever; WO 97/49805, WO 01/21817, WO 03/035694, WO 03/054016 y WO 03/055527 del Vlaams Instituut voor Biotechnologie (VIB);

WO 03/050531 de Algonomics N.V. y solicitante; WO 01/90190 del National Research Council of Canada; WO 03/025020 (= EP 1 433 793) del Institute of Antibodies; así como WO 04/041867, WO 04/041862, WO 04/041865, WO 04/041863, WO 04/062551 del solicitante applicant y las solicitudes de patente publicadas adicionales del solicitante; Hamers-Casterman et al., Nature 1993 Junio 3; 363 (6428): 446-8; Davies and Riechmann, FEBS Lett. 1994 Feb 21; 339(3): 285-90; Muyldermans et al., Protein Eng. 1994 Sep; 7(9): 1129-3; Davies and Riechmann, Biotechnology (NY) 1995 Mayo; 13(5): 475-9; Gharoudi et al., 9th Forum of Applied Biotechnology, Med. Fac. Landbouw Univ. Gent. 1995; 60/4a parte I: 2097-2100; Davies and Riechmann, Protein Eng. 1996 Junio; 9(6): 531- 7; Desmyter et al., Nat Struct Biol. 1996 Septiembre; 3(9): 803-11; Sheriff et al., Nat Struct Biol. 1996 Septiembre; 3(9): 733-6; Spinelli et al., Nat Struct Biol. 1996 Septiembre; 3(9): 752-7; Arbabi Ghahroudi et al., FEBS Lett. 1997 Septiembre 15; 414(3): 521-6; Vu et al., Mol Immunol. 1997 Noviembre-Diciembre; 34(16-17): 1121-31; Atarhouch et al., Journal of Camel Practice and Research 1997; 4: 177-182; Nguyen et al., J. Mol. Biol. 1998 Enero 23; 275(3): 413-8; Lauwereys et al., EMBO J. 1998 Julio 1; 17(13): 3512-20; Frenken et al., Res Immunol. 1998 Julio- Agosto; 149(6):589-99; Transue et al., Proteins 1998 Septiembre 1; 32(4): 515-22; Muyldermans and

Lauwereys, J. Mol. Recognit. 1999 Marzo-Abril; 12 (2): 131-40; van der Linden et al., Biochim. Biophys. Acta 1999 Abril 12; 1431(1): 37-46.; Decanniere et al., Structure Fold. Des. 1999 Abril 15; 7(4): 361-70; Ngyuen et al., Mol. Immunol. 1999 Junio; 36(8): 515-24; Woolven et al., Immunogenetics 1999 Octubre; 50 (1-2): 98-101; Riechmann and Muyldermans, J. Immunol. Methods 1999 Diciembre 10; 231 (1-2): 25-38; Spinelli et al., Biochemistry 2000 Febrero 15; 39(6): 1217-22; Frenken et al., J. Biotechnol. 2000 Febrero 28; 78(1): 11-21; Nguyen et al., EMBO J. 2000 Marzo 1; 19(5): 921-30; van der Linden et al., J. Immunol. Methods 2000 Junio 23; 240 (1-2): 185-95; Decanniere et al., J. Mol. Biol. 2000 Junio 30; 300 (1): 83-91; van der Linden et al., J. Biotechnol. 2000 Julio 14; 80(3): 261-70; Harmsen et al., Mol. Immunol. 2000 Agosto; 37(10): 579-90; Perez et al., Biochemistry 2001 Enero 9; 40(1): 74-83; Conrath et al., J. Biol. Chem. 2001 Marzo 9; 276 (10): 7346-50; Muyldermans et al., Trends Biochem Sci. 2001 Abril; 26(4):230-5; Muyldermans S., J. Biotechnol. 2001 Junio; 74 (4): 277-302; Desmyter et al., J. Biol. Chem. 2001 Julio 13; 276 (28): 26285-90; Spinelli et al., J. Mol. Biol. 2001 Agosto 3; 311 (1): 123-9; Conrath et al., Antimicrob Agents Chemother. 2001 Octubre; 45 (10): 2807-12; Decanniere et al., J. Mol. Biol. 2001 Octubre 26; 313(3): 473-8; Nguyen et al., Adv Immunol. 2001; 79: 261-

96; Muruganandam et al., FASEB J. 2002 Febrero; 16 (2): 240-2; Ewert et al., Biochemistry 2002 Marzo 19; 41 (11): 3628-36; Dumoulin et al., Protein Sci. 2002 Marzo; 11 (3): 500-15; Cortez- Retamozo et al., Int. J. Cancer. 2002 Marzo 20; 98 (3): 456-62; Su et al., Mol. Biol. Evol. 2002 Marzo; 19 (3): 205-15; van der Vaart JM., Methods Mol Biol. 2002; 178: 359-66; Vranken et al., Biochemistry 2002 Julio 9; 41 (27): 8570-9; Nguyen et al., Immunogenetics 2002 Abril; 54 (1): 39-47; Renisio et al., Proteins 2002 Junio 1; 47 (4): 546-55; Desmyter et al., J. Biol. Chem. 2002 Junio 28; 277 (26): 23645-50; Ledeboer et al., J. Dairy Sci. 2002 Junio; 85 (6): 1376-82; De Genst et al., J. Biol. Chem. 2002 Agosto 16; 277 (33): 29897-907; Ferrat et al., Biochem. J. 2002 Septiembre 1; 366 (Pt 2): 415-22; Thomassen et al., Enzyme and Microbial Technol. 2002; 30: 273-8; Harmsen et al., Appl. Microbiol. Biotechnol. 2002 Diciembre; 60 (4): 449-54; Jobling et al., Nat Biotechnol. 2003 Enero; 21 (1): 77-80; Conrath et al., Dev. Comp. Immunol. 2003 Febrero; 27 (2): 87-103; Pleschberger et al., Bioconjug. Chem. 2003 Marzo-Abril; 14 (2): 440-8; Lah et al., J. Biol. Chem. 2003 Abril 18; 278 (16): 14101-1 1; Nguyen et al., Immunology. 2003 Mayo; 109 (1): 93-101; Joosten et al., Microb. Cell Fact. 2003 Enero 30; 2 (1): 1; Li et al., Proteins 2003 Julio 1; 52 (1): 47-50; Loris et al., Biol Chem. 2003 Julio 25; 278 (30): 28252-7; van Koningsbruggen et al., J.

Immunol. Methods. 2003 Agosto; 279 (1-2): 149-61; Dumoulin et al., Nature. 2003 Agosto 14; 424 (6950): 783-8; Bond et al., J. Mol. Biol. 2003 Septiembre 19; 332 (3): 643-55; Yau et al., J. Immunol. Methods. 2003 Octubre 1; 281 (1-2): 161-75; Dekker et al., J. Virol. 2003 Noviembre; 77 (22): 12132-9; Meddeb-Mouelhi et al., Toxicon. 2003 Diciembre; 42 (7): 785-91; Verheesen et al., Biochim. Biophys. Acta 2003 Diciembre 5; 1624 (1-3): 21-8; Zhang et al., J Mol Biol. 2004 Enero 2; 335 (1): 49-56; Stijlemans et al., J Biol Chem. 2004 Enero 9; 279 (2): 1256-61; Cortez-Retamozo et al., Cancer Res. 2004 Abril 15; 64 (8): 2853-7; Spinelli et al., FEBS Lett. 2004 Abril 23; 564 (1-2): 35-40; Pleschberger et al., Bioconjug. Chem. 2004 Mayo-Junio; 15 (3): 664-71; Nicaise et al., Protein Sci. 2004 Julio; 13 (7): 1882-91; Omidfar et al., Tumour Biol. 2004 Julio-Agosto; 25 (4): 179-87; Omidfar et al., Tumour Biol. 2004 Septiembre-Diciembre; 25(5-6): 296-305; Szynol et al., Antimicrob Agents Chemother. 2004 Septiembre; 48(9):3390-5; Saerens et al., J. Biol. Chem. 2004 Diciembre 10; 279 (50): 51965-72; De Genst et al., J. Biol. Chem. 2004 Diciembre 17; 279 (51): 53593-601; DoUc et al., Appl. Environ. Microbiol. 2005 Enero; 71(1): 442-50; Joosten et al., Appl Microbiol Biotechnol. 2005 Enero; 66(4): 384-92; Dumoulin et al., J. Mol. Biol. 2005 Febrero 25; 346 (3): 773-88; Yau et al., J Immunol Methods. 2005 Febrero; 297 (1-2): 213-24;

De Genst et al., J. Biol. Chem. 2005 Abril 8; 280 (14): 14114-21; Huang et al., Eur. J. Hum. Genet. 2005 Abril 13; Dolk et al., Proteins. 2005 Mayo 15; 59 (3): 555-64; Bond et al., J. Mol. Biol. 2005 Mayo 6;348(3):699-709; Zarebski et al., J. Mol. Biol. 2005 Abril 21; [E-publicación antes de impresión].

De conformidad con la terminología usada en las referencias anteriores, los dominios variables presentes en anticuerpos de cadena pesada que se presentan naturalmente también serán referidos como "*dominios* V_{HH}", para distinguirlos de los dominios variables de cadena presada que están presentes en anticuerpos de 4-cadenas convencionales (los cuales serán referidos posteriormente como "*dominios* V_H") y de los dominios variables de cadena ligera que están presentes en los anticuerpos de 4 cadenas convencionales (los cuales serán referidos posteriormente como "*dominios* V_L").

Como se mencionó en la técnica previa referida anteriormente, los dominios V_{HH} tienen un número de características estructurales únicas y propiedades funcionales las cuales hacen a los dominios V_{HH} aislados (así como los Nanocuerpos a base de estos, los cuales comparten estas características estructurales y propiedades funcionales con los dominios V_{HH} que se presentan naturalmente) y proteínas que contienen los mismos

altamente ventajosos para el uso como proteínas o dominios de enlace de antígeno funcionales. En particular, y sin que se limite a esto, los dominios V_{HH} (los cuales se han "diseñado" por la naturaleza para enlazar funcionalmente un antígeno sin la presencia de, y sin alguna interacción con, un dominio variable de cadena ligera) y los Nanocuerpos pueden funcionar como una unidad, dominio o proteína estructural de enlace de antígeno funcional, relativamente pequeña, única. Esto distingue a los dominios V_{HH} de los dominios V_H y V_L de los anticuerpos de 4 cadenas convencionales, los cuales por si mismo son generalmente no adecuados para la aplicación práctica como proteínas o dominios de enlace de antígeno, sino necesitan ser combinados en alguna forma u otra para proporcionar una unidad de enlace de antígeno funcional (como en por ejemplo fragmentos de anticuerpo convencionales tales como fragmentos Fab; en fragmentos ScFv, los cuales consisten de un dominio V_H covalentemente enlazado a un dominio V_L).

Debido a estas propiedades únicas, el uso de dominios V_{HH} y Nanocuerpos como proteínas de enlace de antígeno únicas o como dominios de enlace de antígeno (es decir, como parte de una proteína o polipéptido mayor) ofrece un número de ventajas significativas sobre el uso de dominios V_H y V_L convencionales, scFv's o fragmentos de anticuerpo convencionales (tales como fragmentos Fab o

$F(ab')_2$):

- solamente un dominio único es requerido para enlazar un antígeno con alta afinidad y con alta selectividad, de modo que no hay necesidad de tener dos dominios separados presentes, ni para asegurar que estos dos dominios están presentes en la conformación y configuración espacial recta (es decir, a través del uso de enlazantes especialmente diseñados, como con scFv's);
- los dominios V_{HH} y Nanocuerpos se pueden expresar de un gen único y no requieren doblez post-traduccional o modificaciones;
- los dominios V_{HH} y Nanocuerpos fácilmente se pueden fabricar en formatos multivalentes y multiespecíficos (como se discute adicionalmente en la presente);
- los dominios V_{HH} y Nanocuerpos son altamente solubles y no tienen una tendencia a agregarse (como con los dominios de enlace de antígeno derivados de ratón por Ward et al., Nature, Vol. 341, 1989, p. 544);
- los dominios V_{HH} y Nanocuerpos son altamente estable al calor, pH, proteasas y otros agentes o condiciones desnaturalizadas (véase por ejemplo Ewert et al, supra);
- los dominios V_{HH} y Nanocuerpos son fáciles y relativamente baratos de preparar, aún en una escala

requerida para producción. Por ejemplo, los dominios V_{HH}, Nanocuerpos y proteínas/polipéptidos que contienen los mismos se pueden producir usando fermentación microbiana (por ejemplo como se describe adicionalmente posteriormente) y no requieren el uso de sistemas de expresión de mamífero, como con, por ejemplo, fragmentos de anticuerpo convencional;

- los dominios V_{HH} y Nanocuerpos son relativamente pequeños (aproximadamente 15 kDa, ó 10 veces menor que una IgG convencional) comparados con los anticuerpos de 4 cadenas convencionales y fragmentos de enlace de antígeno de los mismos, y por lo tanto muestran alta (mayor) penetración en los tejidos (incluyendo pero no limitado a tumores sólidos y otros tejidos densos) que tales anticuerpos de 4 cadenas convencionales y fragmentos de enlace de antígeno de los mismos;
- los dominios V_{HH} y Nanocuerpos pueden mostrar propiedades de enlace de cavidad así llamadas (inter alia debido a su bucle CDR3 extendido, comparado con los dominios V_H convencionales) y por lo tanto también pueden acceder a objetivos y epítopes no accesibles por los anticuerpos de 4 cadenas convencionales y fragmentos de enlace de antígeno de los mismos. Por ejemplo, se ha mostrado que los dominios V_{HH} y Nanocuerpos pueden inhibir las enzimas (véase por

ejemplo WO 97/49805; Transue et al., (1998), supra; Lauwereys et al., (1998), supra.

Como se mencionó anteriormente, la invención generalmente se refiere a Nanocuerpos dirigidos contra TNF-alfa, así como a polipéptidos que comprenden o esencialmente consisten de uno o más de tales Nanocuerpos, que se pueden usar para propósitos profilácticos, terapéuticos y/o de diagnóstico descritos posteriormente y en WO 04/041862.

También como se mencionó y describe adicionalmente posteriormente, la invención adicionalmente se refiere a ácidos nucleicos que codifican tales Nanocuerpos y polipéptidos, a métodos para preparar tales Nanocuerpos y polipéptidos, a células hospedadoras que expresan o son capaces de expresar tales Nanocuerpos o polipéptidos, a usos de tales Nanocuerpos, polipéptidos, ácidos nucleicos o células hospedadoras, y a composiciones que comprenden tales Nanocuerpos, polipéptidos, ácidos nucleicos o células hospedadoras.

Generalmente, se deberá señalar que el término Nanocuerpo como se usa en la presente en su sentido más amplio no se limita a una fuente biológica específica o a un método específicos de preparación. Por ejemplo, como se discutirá con más detalle posteriormente, los Nanocuerpos de la invención se pueden obtener (1) aislando el dominio

V_{HH} de un anticuerpo de cadena pesada que se presenta naturalmente; (2) por expresión de una secuencia de nucleótido que codifica un dominio V_{HH} que se presenta naturalmente; (3) por "humanización" (como se describe posteriormente) de un dominio V_{HH} que se presenta naturalmente o por expresión de un ácido nucleico que codifica un dominio V_{HH} humanizado; (4) por "camelización" (como se describe posteriormente) de un dominio V_H que se presenta naturalmente de cualquier especie animal, en particular una especie de mamífero, tal como de un ser humano, o por expresión de un ácido nucleico que codifica tal dominio V_H camelizado; (5) por "camelización" de un "anticuerpo de dominio" o "Dab" como se describe por Ward et al (supra), o por expresión de un ácido nucleico tal como un dominio V_H camelizado; (6) usando técnicas sintéticas o semi-sintéticas para preparar proteínas, polipéptidos u otras secuencias de aminoácido; (7) preparando un ácido nucleico que codifica un Nanocuerpo usando técnicas para síntesis de ácido nucleico, seguido por expresión del ácido nucleico así obtenido, y/o (8) por cualquier combinación de los anteriores. Los métodos y técnicas adecuadas para realizar lo anterior serán claros para la persona experta basado en la descripción en la presente y por ejemplo incluyen los métodos y técnicas descritos con más detalles posteriormente.

Sin embargo, de conformidad con una modalidad específica, los Nanocuerpos de la invención no tienen una secuencia de aminoácido que es exactamente la misma como (es decir como un grado de identidad de secuencia de 100% con) la secuencia de aminoácido de un dominio V_H que se presenta naturalmente, tal como la secuencia de aminoácido de un dominio V_H que se presenta naturalmente de un mamífero, y en particular de un ser humano.

Una clase particularmente preferida de Nanocuerpos de la invención comprende Nanocuerpos con una secuencia de aminoácido que corresponde a la secuencia de aminoácido de un dominio V_{HH} que se presenta naturalmente, pero que se ha "humanizado", es decir reemplazando uno o más residuos de aminoácido en la secuencia de aminoácido de la secuencia V_{HH} que se presenta naturalmente por uno o más de los residuos aminoácido que se presentan en las posiciones correspondientes en un dominio V_H de un anticuerpo de 4 cadenas convencional de un ser humano (por ejemplo indicado anteriormente). Esto se puede realizar en una manera conocida per se, la cual será clara para la persona experta, por ejemplo en la base de la descripción adicional posteriormente y la técnica previa en la humanización referida en la presente. De nuevo, se deberá señalar que tales Nanocuerpos humanizados de la invención se pueden obtener en cualquier manera conocida per ser (es

decir como se indicó bajo los puntos (1)-(8) anteriores) y por consiguiente no son estrictamente limitados a polipéptidos que se han obtenido usando un polipéptido que comprende un dominio V_{HH} que se presenta naturalmente como un material de partida.

Una sustitución humanizante preferida, pero no limitante de Nanocuerpos que pertenecen al grupo 103 P,R,S y/o el grupo GLEW (como se define en la presente) es 108Q a 108L.

Otra clase particularmente preferida de Nanocuerpos de la invención comprende Nanocuerpos con una secuencia de aminoácido que corresponde a la secuencia de aminoácido de un dominio V_H que se presenta naturalmente que se ha "camelizado", es decir reemplazando uno o más residuos aminoácido en la secuencia de aminoácido de un dominio V_H que se presenta naturalmente de un anticuerpo de 4 cadenas convencional por uno o más de los residuos aminoácido que se presentan en las posiciones correspondientes en un dominio V_{HH} de un anticuerpo de cadena pesada. Esto se puede realizar en una manera conocida per se, la cual será clara para la persona experta, por ejemplo en la base de la descripción adicional posteriormente. También se hace referencia a WO 94/04678. Tal camelización preferiblemente puede ocurrir en posiciones de aminoácido las cuales están presentes en la

interfase V_H-V_L y en los residuos distintivos camélidos así llamados (véase por ejemplo también WO 94/04678), como también se menciona posteriormente. Preferiblemente, el dominio V_H o secuencia que se usa como un material de partida o punto de partida para generar o diseñar el Nanocuerpo camelizado preferiblemente es una secuencia de V_H de un mamífero, más preferiblemente la secuencia de V_H de un ser humano. Sin embargo, se deberá señalar que tales Nanocuerpos camelizados de la invención se pueden obtener en cualquier manera conocida per se (es decir como se indica bajo los puntos (1)-(8) anteriores) y por consiguiente no se limitan estrictamente a polipéptidos que se han obtenido usando un polipéptido que comprende un dominio V_H que se presenta naturalmente como un material de partida.

Por ejemplo, de nuevo como se describe adicionalmente posteriormente, tanto "humanización" como "camelización" se pueden realizar proporcionando una secuencia de nucleótido que codifica tal dominio V_{HH} o dominio V_H que se presenta naturalmente, respectivamente, y luego cambiando, en una manera conocida per se, uno o más codones en la secuencia de nucleótido de modo que la nueva secuencia de nucleótido codifica un Nanocuerpo humanizado o camelizado de la invención, respectivamente, y luego expresando la secuencia de nucleótido así obtenida en una

manera conocida per se para proporcionar el Nanocuerpo deseado de la invención. Alternativamente, basado en la secuencia de aminoácido de un dominio V_{HH} o dominio V_H que se presenta naturalmente, respectivamente, la secuencia de aminoácido del Nanocuerpo humanizado o camelizado deseado de la invención, respectivamente, se puede diseñar y luego sintetizar *de novo* usando técnicas para síntesis de péptido conocidas per se. Además, basado en la secuencia de aminoácido o secuencia de nucleótido de un dominio V_{HH} o dominio V_H que se presenta naturalmente, respectivamente, una secuencia de nucleótido que codifica el Nanocuerpo humanizado o camelizado deseado de la invención, respectivamente, se pueden diseñar y luego sintetizar *de novo* usando técnicas para síntesis de ácido nucleico conocidas per se, después de lo cual la secuencia de nucleótido así obtenida se puede expresar en una manera conocida per se para proporcionar el Nanocuerpo deseado de la invención.

Otras formas y técnicas adecuadas para obtener Nanocuerpos de la invención y/o secuencias de nucleótido y/o ácidos nucleicos que codifican las mismas, partiendo de (la secuencia de aminoácido de) dominios V_H que se presentan naturalmente o preferiblemente dominios V_{HH} y/o de las secuencias de nucleótido y/o secuencias de ácido nucleico que codifican las mismas serán claras para la

persona experta, y por ejemplo pueden comprender combinar una o más secuencias de aminoácido y/o secuencias de nucleótido de dominios V_H que se presentan naturalmente (tales como una o más FR's y/o CDR's) con una o más secuencias de aminoácido y/o secuencias de nucleótido de dominios V_{HH} que se presentan naturalmente (tal como una o más FR's o CDR's), en una manera adecuada para proporcionar (una secuencia de nucleótido o ácido nucleico codificante) un Nanocuerpo de la invención.

De conformidad con un aspecto preferido, pero no limitante del aspecto de la invención, un Nanocuerpo en su sentido más amplio se puede definir generalmente como un polipéptido que comprende:

a) una secuencia de aminoácido que está comprendida de cuatro regiones/secuencias de estructura interrumpidas por tres regiones/secuencias determinantes de complementariedad, en las cuales el residuo aminoácido en la posición 108 de conformidad con la numeración Kabat es Q;

y/o:

b) una secuencia de aminoácido que está comprendida de cuatro regiones/secuencias de estructura interrumpidas por tres regiones/secuencias determinantes de complementariedad, en las cuales el residuo aminoácido en la posición 44 de conformidad con la numeración

Kabat es E y en las cuales el residuo aminoácido en la posición 45 de conformidad con la numeración Kabat es un R;

y/o:

c) una secuencia de aminoácido que está comprendida de cuatro regiones/secuencias de estructura interrumpidas por tres regiones/secuencias determinantes de complementariedad, en las cuales el residuo aminoácido en la posición 103 de conformidad con la numeración Kabat es elegido del grupo que consiste de P, R y S, y es en particular elegido del grupo que consiste de R y S.

Por consiguiente, en un primer aspecto preferido, pero no limitante, un Nanocuerpo de la invención puede tener la estructura

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

en la cual FR1 a FR4 se refieren a regiones de estructura 1 a 4, respectivamente, y en las cuales CDR1 a CDR3 se refieren a las regiones determinantes de complementariedad 1 a 3, respectivamente, y en las cuales

i) el residuo aminoácido en la posición 108 de conformidad con la numeración Kabat es Q;

y/o en las cuales:

ii) el residuo aminoácido en la posición 44 de conformidad con la numeración Kabat es E y en la cual el residuo

aminoácido en la posición 45 de conformidad con la numeración Kabat es un R;

y/o en las cuales:

iii) el residuo aminoácido en la posición 103 de conformidad con la numeración Kabat se elige del grupo que consiste de P, R y S, y es en particular elegido del grupo que consiste de R y S;

y en las cuales

iv) CDR1, CDR2 y CDR3 son como se definieron anteriormente, y son preferiblemente como se definen de conformidad con una de las definiciones preferidas anteriores, y son más preferiblemente como se definen de conformidad con una o más de las definiciones preferidas anteriores.

En particular, un Nanocuerpo contra TNF-alfa de conformidad con la invención puede tener la estructura:

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

en la cual FR1 a FR4 se refieren a regiones de estructura 1 a 4, respectivamente, y en las cuales CDR1 a CDR3 se refieren a las regiones determinantes de complementariedad 1 a 3, respectivamente, y en las cuales

i) el residuo aminoácido en la posición 108 de conformidad con la numeración Kabat es Q;

y/o en las cuales:

ii) el residuo aminoácido en la posición 44 de conformidad

con la numeración Kabat es E y en la cual el residuo aminoácido en la posición 45 de conformidad con la numeración Kabat es un R;

y/o en las cuales:

iii) el residuo aminoácido en la posición 103 de conformidad con la numeración Kabat se elige del grupo que consiste de P, R y S, y es en particular elegido del grupo que consiste de R y S;

y en las cuales

iv) CDR1, CDR2 y CDR3 son como se definieron anteriormente, y son preferiblemente como se definen de conformidad con una de las definiciones preferidas anteriores, y son más preferiblemente como se definen de conformidad con una de las definiciones más preferidas anteriores.

En particular, de conformidad con un aspecto preferido, pero no limitante del aspecto de la invención, un Nanocuerpo generalmente se puede definir como un polipéptido que comprende una secuencia de aminoácido que está comprendida de cuatro regiones/secuencias de estructura interrumpidas por tres regiones/secuencias determinantes de complementariedad, en las cuales;

a-1) el residuo aminoácido en la posición 44 de conformidad con la numeración Kabat se elige del grupo que consiste de G, E, D, G, Q, R, S, L; y preferiblemente

se elige del grupo que consiste de G, E o Q; y

a-2) el residuo aminoácido en la posición 45 de conformidad con la numeración Kabat se elige del grupo que consiste de L, R o C; y preferiblemente se elige del grupo que consiste de L o R; y

a-3) el residuo aminoácido en la posición 103 de conformidad con la numeración Kabat se elige del grupo que consiste de W, R o S; y preferiblemente es W o R, y es muy preferiblemente W;

a-4) el residuo aminoácido en la posición 108 de conformidad con la numeración Kabat es Q;

o en las cuales:

b-1) el residuo aminoácido en la posición 44 de conformidad con la numeración Kabat se elige del grupo que consiste de E y Q; y

b-2) el residuo aminoácido en la posición 45 de conformidad con la numeración Kabat es R; y

b-3) el residuo aminoácido en la posición 103 de conformidad con la numeración Kabat se elige del grupo que consiste de W, R y S; y es preferiblemente W;

b-4) el residuo aminoácido en la posición 108 de conformidad con la numeración Kabat se elige del grupo que consiste de Q y L; y es preferiblemente Q;

o en las cuales:

c-1) el residuo aminoácido en la posición 44 de conformidad

con la numeración Kabat se elige del grupo que consiste de G, E, D, Q, R, S y L; y preferiblemente se elige del grupo que consiste de G, E y Q; y

c-2) el residuo aminoácido en la posición 45 de conformidad con la numeración Kabat se elige del grupo que consiste de L, R y C; y preferiblemente se elige del grupo que consiste de L y R; y

c-3) el residuo aminoácido en la posición 103 de conformidad con la numeración Kabat se elige del grupo que consiste de P, R y S; y en particular se elige del grupo que consiste de R y S; y

c-4) el residuo aminoácido en la posición 108 de conformidad con la numeración Kabat se elige del grupo que consiste de Q y L; y es preferiblemente Q.

Por consiguiente, en otro aspecto preferido, pero no limitante, un Nanocuerpo de la invención puede tener la estructura

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

en la cual FR1 a FR4 se refieren a regiones de estructura 1 a 4, respectivamente, y en las cuales CDR1 a CDR3 se refieren a las regiones determinantes de complementariedad 1 a 3, respectivamente, y en las cuales:

i) el residuo aminoácido en la posición 44 de conformidad con la numeración Kabat se elige del grupo que consiste de G, E, D, G, Q, R, S, L; y preferiblemente

se elige del grupo que consiste de G, E o Q;

y en las cuales:

ii) el residuo aminoácido en la posición 45 de conformidad con la numeración Kabat se elige del grupo que consiste de L, R o C; y preferiblemente se elige del grupo que consiste de L o R;

y en las cuales:

iii) el residuo aminoácido en la posición 103 de conformidad con la numeración Kabat se elige del grupo que consiste de W, R o S; y preferiblemente es W o R, y es más preferiblemente W;

y en las cuales

iv) el residuo aminoácido en la posición 108 de conformidad con la numeración Kabat es Q;

y en las cuales:

v) CDR1, CDR2 y CDR3 son como se definieron anteriormente, y son preferiblemente como se definen de conformidad con una de las definiciones preferidas anteriores, y son más preferiblemente como se definen de conformidad con una de las definiciones más preferidas anteriores.

En otro aspecto preferido, pero no limitante, un Nanocuerpo de la invención puede tener la estructura

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

en la cual FR1 a FR4 se refieren a regiones de estructura 1

a 4, respectivamente, y en las cuales CDR1 a CDR3 se refieren a las regiones determinantes de complementariedad 1 a 3, respectivamente, y en las cuales:

i) el residuo aminoácido en la posición 44 de conformidad con la numeración Kabat se elige del grupo que consiste de E y Q;

y en las cuales:

ii) el residuo aminoácido en la posición 45 de conformidad con la numeración Kabat es R;

y en las cuales:

iii) el residuo aminoácido en la posición 103 de conformidad con la numeración Kabat se elige del grupo que consiste de W, R y S; y preferiblemente es W;

y en las cuales

iv) el residuo aminoácido en la posición 108 de conformidad con la numeración Kabat es Q;

y en las cuales:

v) CDR1, CDR2 y CDR3 son como se definieron anteriormente, y son preferiblemente como se definen de conformidad con una de las definiciones preferidas anteriores, y son más preferiblemente como se definen de conformidad con una de las definiciones más preferidas anteriores.

En otro aspecto preferido, pero no limitante, un Nanocuerpo de la invención puede tener la estructura

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

en la cual FR1 a FR4 se refieren a regiones de estructura 1 a 4, respectivamente, y en las cuales CDR1 a CDR3 se refieren a las regiones determinantes de complementariedad 1 a 3, respectivamente, y en las cuales:

i) el residuo aminoácido en la posición 44 de conformidad con la numeración Kabat se elige del grupo que consiste de G, E, D, Q, R, S y L; y preferiblemente se elige del grupo que consiste de G, E y Q;

y en las cuales:

ii) el residuo aminoácido en la posición 45 de conformidad con la numeración Kabat se elige del grupo que consiste de L, R y C; y preferiblemente se elige del grupo que consiste de L y R;

y en las cuales:

iii) el residuo aminoácido en la posición 103 de conformidad con la numeración Kabat se elige del grupo que consiste de P, R y S; y en particular se elige del grupo que consiste de R y S;

y en las cuales

iv) el residuo aminoácido en la posición 108 de conformidad con la numeración Kabat se elige del grupo que consiste de Q y L; preferiblemente es Q;

y en las cuales:

v) CDR1, CDR2 y CDR3 son como se definieron

anteriormente, y son preferiblemente como se definen de conformidad con una de las definiciones preferidas anteriores, y son más preferiblemente como se definen de conformidad con una de las definiciones más preferidas anteriores.

Dos grupos particularmente preferidos, pero no limitantes de los Nanocuerpos de la invención son aquellos de conformidad con a) anterior; de conformidad con I) a a-4) anterior; de conformidad con b) anterior; de conformidad con b-1) a b-4) anteriores; de conformidad con c) anterior; y/o de conformidad con c-1) a c-4) anteriores, en los cuales;

a) los residuos aminoácido en las posiciones 44-47 de conformidad con la numeración Kabat forman la secuencia GLEW (o una secuencia tipo GLEW como se define posteriormente) y el residuo aminoácido en la posición 108 es Q;

en los cuales:

b) los residuos aminoácido en las posiciones 43-46 de conformidad con la numeración Kabat forman la secuencia KERE o KQRE (o una secuencia tipo KERE) y el residuo aminoácido en la posición 108 es Q o L, y preferiblemente es Q.

Por consiguiente, en otro aspecto preferido, pero no limitante, un Nanocuerpo de la invención puede tener la

estructura

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

en la cual FR1 a FR4 se refieren a regiones de estructura 1 a 4, respectivamente, y en las cuales CDR1 a CDR3 se refieren a las regiones determinantes de complementariedad 1 a 3, respectivamente, y en las cuales:

i) los residuos aminoácido en las posiciones 44-47 de conformidad con la numeración Kabat forman la secuencia GLEW (o una secuencia tipo GLEW como se define posteriormente) y el residuo aminoácido en la posición 108 es Q;

y en las cuales:

ii) CDR1, CDR2 y CDR3 son como se definieron anteriormente, y son preferiblemente como se definen de conformidad con una de las definiciones preferidas anteriores, y son más preferiblemente como se definen de conformidad con una de las definiciones más preferidas anteriores.

En otro aspecto preferido, pero no limitante, un Nanocuerpo de la invención puede tener la estructura

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

en la cual FR1 a FR4 se refieren a regiones de estructura 1 a 4, respectivamente, y en las cuales CDR1 a CDR3 se refieren a las regiones determinantes de complementariedad 1 a 3, respectivamente, y en las cuales:

i) los residuos aminoácido en las posiciones 43-46 de conformidad con la numeración Kabat forman la secuencia KERE o KQRE (o una secuencia tipo KERE) y el residuo aminoácido en la posición 108 es Q o L, y preferiblemente es Q;

y en las cuales:

ii) CDR1, CDR2 y CDR3 son como se definieron anteriormente, y son preferiblemente como se definen de conformidad con una de las definiciones preferidas anteriores, y son más preferiblemente como se definen de conformidad con una de las definiciones más preferidas anteriores.

En los Nanocuerpos de la invención en los cuales los residuos aminoácido en las posiciones 43-46 de conformidad con la numeración Kabat forman la secuencia KERE o KQRE, el residuo aminoácido en la posición 37 es más preferiblemente F. En los Nanocuerpos de la invención en los cuales los residuos aminoácido en las posiciones 44-47 de conformidad con la numeración Kabat forman la secuencia GLEW, el residuo aminoácido en la posición 37 se elige del grupo que consiste de Y, H, I, V o F, y es más preferiblemente F.

Por consiguiente, sin que se limite a esto en alguna forma, sobre la base de los residuos aminoácido presentes en las posiciones mencionadas anteriormente, los

Nanocuerpos de la invención generalmente se pueden clasificar sobre la base de los siguientes tres grupos:

a) El "*grupo GLEW*": Nanocuerpos con la secuencia de aminoácido GLEW en las posiciones 44-47 de conformidad con la numeración Kabat y Q en la posición 108 de conformidad con la numeración Kabat. Como se describe adicionalmente en la presente, los Nanocuerpos dentro de este grupo usualmente tienen un V en la posición 37, y pueden tener un W, P, R o S en la posición 103, y preferiblemente tienen un W en la posición 103. El grupo GLEW también comprende algunas secuencias tipo GLEW tales como aquellas mencionadas en la tabla 2 posterior;

b) El "*grupo KERE*": Nanocuerpos con la secuencia de aminoácido KERE o KQRE en las posiciones 43-46 de conformidad con la numeración Kabat y Q o L en la posición 108 de conformidad con la numeración Kabat. Como se describe adicionalmente en la presente, los Nanocuerpos dentro de este grupo usualmente tienen un F en la posición 37, un L o F en la posición 47; y pueden tener un W, P, R o S en la posición 103, y preferiblemente tienen un W en la posición 103;

c) El "*grupo 103 P, R, S*": Nanocuerpos con un P, R o S en la posición 103. Estos Nanocuerpos pueden tener ya sea la secuencia de aminoácido GLEW en las posiciones 44-

47 de la numeración Kabat o la secuencia aminoácido KERE o KQRE en las posiciones 43-46 de conformidad con la numeración Kabat, la última más preferiblemente en combinación con un F en la posición 37 y un L o un F en la posición 47 (como se define para el grupo KERE); y pueden tener Q o L en la posición 108 de conformidad con la numeración Kabat; y preferiblemente tienen Q.

Por consiguiente, en otro aspecto preferido, pero no limitante, un Nanocuerpo de la invención puede ser un Nanocuerpo que pertenece al grupo GLEW (como se define en la presente), y en el cual CDR1, CDR2 y CDR3 son como se definieron anteriormente, y preferiblemente son como se definen de conformidad con una de las definiciones preferidas anteriores, y son más preferiblemente como se definen de conformidad con una de las definiciones más preferidas anteriores.

En otro aspecto preferido, pero no limitante, un Nanocuerpo de la invención puede ser un Nanocuerpo que pertenece al grupo KERE (como se define en la presente), y en el cual CDR1, CDR2 y CDR3 son como se definieron anteriormente, y preferiblemente son como se definen de conformidad con una de las definiciones preferidas anteriores, y son más preferiblemente como se definen de conformidad con una de las definiciones más preferidas anteriores.

Por consiguiente, en otro aspecto preferido, pero no limitante, un Nanocuerpo de la invención puede ser un Nanocuerpo que pertenece al grupo 103 P, R, S (como se define en la presente), y en el cual CDR1, CDR2 y CDR3 son como se definieron anteriormente, y preferiblemente son como se definen de conformidad con una de las definiciones preferidas anteriores, y son más preferiblemente como se definen de conformidad con una de las definiciones más preferidas anteriores.

Además, más generalmente y además de los residuos 108Q, 43E/44R y 103 P, R, S mencionados anteriormente, los Nanocuerpos de la invención pueden contener, en una o más posiciones, en un dominio V_H convencional, que podrían formar (parte de) la interfase V_H/V_L, contener uno o más residuos aminoácido que son más altamente cargados que los residuos aminoácido que se presentan naturalmente en las mismas posiciones en el dominio V_H o V_{HH} que se presenta naturalmente correspondiente, y en particular uno o más residuos aminoácido cargados (como se menciona en la tabla 1).

Tales sustituciones incluyen, pero no se limitan a las secuencias tipo GLEW mencionadas en la tabla 2 posterior; así como las sustituciones que se describen en la Solicitud Internacional WO 00/29004 para "*microcuerpos*" así llamados, por ejemplo un Q en la posición 108 y KLEW en

las posiciones 44-47.

En algunas modalidades de los Nanocuerpos de la invención, el residuo aminoácido en la posición 83 se elige del grupo que consiste de L, M, S, V y W; y preferiblemente es L.

Además, en algunas modalidades de los Nanocuerpos de la invención, el residuo aminoácido en la posición 83 se elige del grupo que consiste de R, K, N, E, I y Q; y es más preferiblemente ya sea K o E (para Nanocuerpos correspondientes a dominios V_{HH} que se presentan naturalmente) o R (para Nanocuerpos "humanizados", como se describe posteriormente). El residuo aminoácido en la posición 84 en algunas modalidades se elige del grupo que consiste de P, A, R, S, D y V, y es más preferiblemente P (para Nanocuerpos correspondientes a dominios V_{HH} que se presentan naturalmente) o R (para Nanocuerpos "humanizados", como se describe posteriormente).

Además, en algunas modalidades de los Nanocuerpos de la invención, el residuo aminoácido en la posición 104 se elige del grupo que consiste de G y D; y es más preferiblemente G.

Colectivamente, los residuos aminoácido en las posiciones 11, 37, 44, 45, 47, 83, 84, 103, 104 y 108, los cuales en los Nanocuerpos son como se mencionó anteriormente, serán referidos en la presente como los

"*Residuos Distintivos*". Los residuos distintivos y los residuos aminoácido en las posiciones correspondientes del dominio VH humano más estrechamente relacionado, se resumen en la tabla 2.

Algunas combinaciones especialmente preferidas de estos residuos distintivos como se presentan en dominios V_{HH} que se presentan naturalmente se mencionan en la tabla 3. Para comparación, los residuos aminoácido correspondientes del V_H3 humano llamado DP-47 se han indicado en letras cursivas.

Tabla 2: Residuos Distintivos en Nanocuerpos

Posición	V_H3 humano	Residuos Distintivos
11	L, V; predominantemente L	L, M, S, V, W; preferiblemente L
37	V, I, F; usualmente V	$F^{(1)}$, Y, H, I o V, preferiblemente $F^{(1)}$ o Y
$44^{(8)}$	G	$G^{(2)}$, $E^{(3)}$, D, Q, R, S, L; preferiblemente $G^{(2)}$, $E^{(3)}$ o Q; más preferiblemente $G^{(2)}$ o $E^{(3)}$.
$45^{(8)}$	L	$L^{(2)}$, $R^{(3)}$, C, I, L, P, Q, V; preferiblemente $L^{(2)}$ o $R^{(3)}$
$47^{(8)}$	W, Y	$W^{(2)}$, $L^{(1)}$ o $F^{(1)}$, A, G, I, M, R, S, o Y; preferiblemente $W^{(2)}$, $L^{(1)}$, $F^{(1)}$ o R
83	R o K; usualmente R	R, $K^{(5)}$, N, $E^{(5)}$, I, M o Q; preferiblemente K o R; más preferiblemente K
84	A, T, D; predominantemente A	$P^{(5)}$, A, L, R, S, D, V; preferiblemente P
103	W	$W^{(4)}$, $P^{(6)}$, $R^{(6)}$, S; preferiblemente W
104	G	G o D; preferiblemente G
108	L, M o T; predominantemente L	Q, $L^{(7)}$ o R; preferiblemente Q o $L^{(7)}$

Notas:

(1): En particular, pero no exclusivamente, en combinación con KERE (SEC ID NO: 437) o KQRE (SEC ID NO: 438) en las posiciones 43-46.

(2): Usualmente como GLEW (SEC ID NO: 439) en las posiciones 44-47.

(3): Usualmente como KERE o KQRE en las posiciones 43-46, por ejemplo como KEREL (SEC ID NO: 440), FEREF (SEC ID NO:441), KQREL (SEC ID NO: 442), KQREF (SEC ID NO: 443) o KEREG (SEC ID NO: 444) en

las posiciones 43-47. Alternativamente, también las secuencias tales como TERE (SEC ID NO:445) (por ejemplo TEREL (SEC ID NO:446)), KECE (SEC ID NO:447) (por ejemplo KECEL (SEC ID NO:448) o KECER (SEC ID NO:449)), RERE (SEC ID NO:450) (por ejemplo REREG (SEC ID NO:451)), QERE (SEC ID NO:452) (por ejemplo QEREG (SEC ID NO:453)), KGRE (SEC ID NO:454) (por ejemplo KGREG (SEC ID NO:455)), KDRE (SEC ID NO:456) (por ejemplo KDREV (SEC ID NO:457)) son posibles. Algunas otras secuencias posibles, pero menos preferidas incluyen por ejemplo DECKL (SEC ID NO:458) y NVCEL (SEC ID NO:459).

(4): Tanto con GLEW en las posiciones 44-47 como KERE o KQRE en las posiciones 43-46.

(5): Frecuentemente como KP o EP en las posiciones 83-84 de dominios V_{HH} que se presentan naturalmente.

(6): En particular, pero no exclusivamente, en combinación con GLEW en las posiciones 44-47.

(7): Con la condición que cuando las posiciones 44-47 son GLEW, la posición 108 siempre es Q.

(8): El grupo GLEW también contiene secuencias tipo GLEW en las posiciones 44-47, tal como por ejemplo GVEW (SEC ID NO:460), EPEW (SEC ID NO:461), GLER (SEC ID NO:462), DQEW (SEC ID NO:463), DLEW (SEC ID NO:464), GIEW (SEC ID NO:465), ELEW (SEC ID NO:466), GPEW (SEC ID NO:467), EWLP (SEC ID NO:467), GPER (SEC ID NO:469), GLER (SEC ID NO:470) y ELEW.

Tabla 3: Algunas combinaciones preferidas de Residuos Distintivos en Nanocuerpos que se presentan naturalmente

Para humanización de estas combinaciones, se hace referencia a la especificación.

	11	**37**	**44**	**45**	**47**	**83**	**84**	**103**	**104**	**108**
DP-47 (humano)	*M*	*V*	*G*	*L*	*W*	*R*	*A*	*W*	*G*	*L*
grupo "KERE"	L	F	E	R	L	K	P	W	G	Q
	L	F	E	R	F	E	P	W	G	Q
	L	F	E	R	F	K	P	W	G	Q
	L	Y	Q	R	L	K	P	W	G	Q
	L	F	L	R	V	K	P	Q	G	Q
	L	F	Q	R	L	K	P	W	G	Q
	L	F	E	R	F	K	P	W	G	Q
grupo "GLEW"	L	V	G	L	W	K	S	W	G	Q
	M	V	G	L	W	K	P	R	G	Q

En los Nanocuerpos, cada residuo aminoácido en cualquier otra posición que los Residuos Distintivos puede ser cualquier residuo aminoácido que naturalmente se presenta en la posición correspondiente (de conformidad con la numeración Kabat) de un dominio V_{HH} que se presenta naturalmente.

Tales residuos aminoácido serán claros para la persona experta. Las tablas 4-7 mencionan algunos residuos no limitantes que pueden estar presentes en cada posición (de conformidad con la numeración Kabat) de la FR1, FR2, FR3 y FR4 de dominios V_{HH} que se presentan naturalmente. Para cada posición, el residuo aminoácido que más frecuentemente se presenta en cada posición de un dominio V_{HH} que se presenta naturalmente (y el cual es el residuo aminoácido más preferido para la posición en un Nanocuerpo) se indica en negrillas; y otros residuos aminoácido preferidos para cada posición se han subrayado (nota: el número de residuos aminoácido que se encuentran en las posiciones 26-30 de dominios V_{HH} que se presentan naturalmente soporta la hipótesis que fundamenta la numeración Chothia (supra) que los residuos en esta posiciones ya forman parte de CDR1).

En las tablas 4-7, algunos de los residuos no limitantes que pueden estar presentes en cada posición de un dominio V_H3 humano también se han mencionado. De nuevo,

para cada posición, el residuo aminoácido que más frecuentemente se presenta en cada posición de un dominio V_H3 humano que se presenta naturalmente se indica en negrillas; y otros residuos aminoácido preferidos se han subrayado.

Tabla 4: Ejemplos no limitantes de residuos aminoácido en FR1 (para las notas al pie, véase las notas al pie de la tabla 2)

Pos.	Residuos aminoácido:	
	V_H3 humano	*V_{HH}'s Camélidos*
1	**E**, Q	**Q**, A, E, D, H, R
2	**V**	**V**, A, E, G, L, M, Q
3	**Q**	**Q**, K, E, H, P, R, Y
4	**L**	**L**, F, P, R, V
5	**V**, L	**Q**, E, L, V, M, P, A, I
6	**E**	**E**, D, Q, A, H
7	**S**, T	**S**, F, H
8	**G**, R	**G**, A, R
9	**G**	**G**, E
10	**G**, V	**G**, D, R, A, E, N, T, V
11	**Residuo distintivo**: L, M, S, V, W, F, N, P, T, Y; preferiblemente L	
12	**V**, I	**V**, A, G, M
13	**Q**, K, R	**Q**, E, K, D, G, A, H, L, N, P, R, T
14	**P**	**A**, Q, A, G, P, T, V, E, F, I, N, S
15	**G**	**G**
16	**G**, R	**G**, A, E, D, N, P, R, S, V, W
17	**S**	**S**, F, T, N, P, A, C
18	**L**	**L**, V, M, Q, R
19	**R**, K	**R**, K, L, N, S, T, A, F, G, I, M, Q
20	**L**	**L**, F, I, V, M, S
21	**S**	**S**, F, T, G, H, P, A
22	**C**	**C**
23	**A**, T	**A**, D, P, S, T, V, E, G, I, L, Q, R
24	**A**	**A**, I, S, T, V, C, E, F, G, L, N, P, Q, Y

Tabla 4: Ejemplos no limitantes de residuos aminoácido en FR1 (continuación)

Pos.	Residuos aminoácido:	
	V_H3 humano	*V_{HH}'s Camélidos*
25	**S**	**S**, A, F, P, T, L, V
26	**G**	**G**, D, E, R, S, V, A, I, M, P, T
27	**F**	S, F, R, L, P, G, N, A, D, E, H, I, K, M, Q, T, V, Y
28	**T**	N, T, E, D, S, I, R, A, G, R, F, Y, L, M, P, V
29	**F**, V	F, L, D, S, I, G, V, A, E, P, T, Y
30	**S**, D, G	N, S, E, G, A, D, M, T, H, I, P, R, V, W

Tabla 5: Ejemplos no limitantes de residuos aminoácido en FR2 (para las notas al pie, véase las notas al pie de la tabla 2)

Pos.	Residuos aminoácido:	
	V_H3 humano	*V_{HH}'s Camélidos*
36	**W**	**W**
37	**Residuo distintivo:** $F^{(1)}$, Y, H, I, A, L, P, S o V, preferiblemente $F^{(1)}$ o Y	
38	**R**	**R**
39	**Q**	**Q**, H, P, R, A, D, G, L, E
40	**A**	**A**, F, G, P, T, V, I, L, N, R, S, Y
41	**P**, S, T	**P**, A, L, S, I, Q, T
42	**G**	**G**, E, D, R, T, V
43	**K**	**K**, D, E, N, Q, R, T, V, A, L, M, S
44	**Residuo distintivo:** $G^{(2)}$, $E^{(3)}$, D, Q, R, S, L, A, F, K, M, N, P, V, W, Y; preferiblemente $G^{(2)}$, $E^{(3)}$ o Q; más preferiblemente $G^{(2)}$ o $E^{(3)}$	
45	**Residuo distintivo:** $L^{(2)}$, $R^{(3)}$, C, I, L, P, Q, V, D, E, G, H, K, T; preferiblemente $L^{(2)}$ o $R^{(3)}$	

46	**E**, V	**E**, D, K, Q, V, A, G, N
47	**Residuo distintivo:** $W^{(2)}$, $L^{(1)}$ o $F^{(1)}$, A, G, I, M, R, S, D, E, H, K, Q, T, V o Y; preferiblemente $W^{(2)}$, $L^{(1)}$, $F^{(1)}$ o R	
48	**V**	**V**, I, L, A, C, E, F, G, H, M, P, Q, R, S, T, V, W, Y
49	**S**, A, G	**A**, S, A, G, T, V, D, E, I, L, Q, R, Y

Tabla 6: Ejemplos no limitantes de residuos aminoácido en FR3 (para las notas al pie, véase las notas al pie de la tabla 2)

Pos.	**Residuos aminoácido:**	
	V_H3 humano	*V_{HH}'s Camélidos*
66	**R**	**R**
67	**F**	**F**, L, V, A, D, I, S, Y
68	**T**	**T**, A, S, D, F, , I, K, N
69	**I**	**I**, M, V, A, F, L, R, S, T
70	**S**	**S**, A, F, E, G, K, P, T, V
71	**R**	**R**, G, I, K, Q, S, T, W, A, F, L, M, N
72	**D**, E	**D**, E, G, N, V, A, H, I, L, Q, S, T
73	**N**, D, G	**N**, D, F, I, K, S, T, Y, A, G, H, L, M, R, V
74	**A**, S	**A**, D, G, N, P, S, T, F, H, I, L, R, V, Y
75	**K**	**K**, A, E, K, L, N, Q, R, D, G, I, M, S, T, V, W
76	**N**, S	**N**, D, K, R, S, T, Y, E, G, H, I, Q
77	S, T, I	**T**, A, E, I, M, S, K, L, N, R, V
78	**L**, A	**V**, L, A, F, G, I, M, E, N, Q, R, S, T, W
79	**Y**, H	**Y**, A, D, F, H, S, T, C, E, I, L, N, V, W
80	**L**	**L**, F, V, M

Tabla 6: Ejemplos no limitantes de residuos aminoácido en FR3 (continuación)

Pos.	Residuos aminoácido:	
	V_H3 humano	*V_{HH}'s Camélidos*
81	**Q**	**Q**, E, R, T, G, H, I, K, L, M, N
82	**M**	**M**, I, L, V, G, P, T
82A	**N**, G	**N**, D, G, H, S, T, A ,E, I, K, R, V
82B	**S**	**S**, N, D, G, R, A, C, E, F, I, K, M, P, T, V
82C	**L**	**L**, P, M, T, V
83	**Residuo distintivo:** R, K[5], N, E[5], I, M, A, D, G, L, Q, S, T o Q; preferiblemente K o R; más preferiblemente K	
84	**Residuo distintivo:** P[5], A, L, R, S, D, V, F, G, H, N, T, Y; preferiblemente P	
85	**E**, G	**E**, D, G, Q, A, N, R, V, Y
86	**D**	**D**, E, F, Y
87	**T**, M	**T**, S, A, C, M
88	**A**	**A**, G, S, D, L, N, P
89	**V**, L	**V**, A, D, I, L, M, N, R, T, E, F, S
90	**Y**	**Y**, F, E, H, N
91	**Y**, H	**Y**, D, F, H, L, S, T, V, C, I, N, R, W
92	**C**	**C**
93	**A**, K, T	**A**, N, G, H, K, R, S, T, V, Y, E, F, I, L, M, Q
94	**K**, R, T	**A**, V, C, F, G, I, L, R, S, D, E, K, M, N, P, Q, T, W, Y, T o K;

Tabla 7: Ejemplos no limitantes de residuos aminoácido en FR4 (para las notas al pie, véase las notas al pie de la tabla 2)

Pos.	Residuos aminoácido:	
	V_H3 humano	*V_{HH}'s Camélidos*
103	**Residuo distintivo:** W[4], P[6], R[6], S, F, G, K, L, N, Q, V, Y; preferiblemente W	
104	**Residuo distintivo:** G, A, R, S, T o D; preferiblemente G	

Pos.	Residuos aminoácido:	
	V_H3 humano	*V_{HH}'s Camélidos*
103	**Residuo distintivo**: W(4), P(6), R(6), S, F, G, K, L, N, Q, V, Y; preferiblemente W	
104	**Residuo distintivo**: G, A, R, S, T o D; preferiblemente G	
105	**Q**, R	**Q**, E, K, P, R, G, H, L, S, V
106	**G**	**G**
107	**T**	**T**, A, I, N, P
108	**Residuo distintivo**: Q, L(7), E, H, N, P, T o R; preferiblemente Q o L(7)	
109	**V**	**V**
110	**T**	**T**, I, A
111	**V**	**V**, A, I, G
112	**S**	**S**, F, A, L, P, T, Y
113	**S**	**S**, A, L, P, F, T

Por consiguiente en otro aspecto preferido, pero no limitante, un Nanocuerpo de la invención puede tener la estructura

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

en la cual FR1 a FR4 se refieren a regiones de estructura 1 a 4, respectivamente, y en las cuales CDR1 a CDR3 se refieren a las regiones determinantes de complementariedad 1 a 3, respectivamente, y en las cuales:

i) los residuos distintivos son como se definieron anteriormente;

y en las cuales:

ii) CDR1, CDR2 y CDR3 son como se definieron anteriormente, y son preferiblemente como se definen de conformidad con una de las definiciones preferidas anteriores, y son más preferiblemente como se definen de conformidad con una de las definiciones más

889

preferidas anteriores.

En otro aspecto preferido, pero no limitante, un Nanocuerpo de la invención puede tener la estructura

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

en la cual FR1 a FR4 se refieren a regiones de estructura 1 a 4, respectivamente, y en las cuales CDR1 a CDR3 se refieren a las regiones determinantes de complementariedad 1 a 3, respectivamente, y en las cuales:

i) FR1 se elige del grupo que consiste de la secuencia de aminoácido:

[1] QVQLQESGGG**X**VQAGGSLRLSCAASG [26] [SEC ID NO: 1]

o del grupo que consiste de secuencias de aminoácido que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en la presente) con la secuencia de aminoácido anterior; en la cual

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 4; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores:

y/o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o solamente 1 "diferencia(s) de aminoácido" (como se define en la presente) con una de las secuencias de aminoácido anteriores, en las cuales:

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 4; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores;

y en las cuales:

ii) FR2 se elige del grupo que consiste de la secuencia de aminoácido:

[36] W**X**RQAPGK**XX**E**X**VA [49] [SEC ID NO: 2]

o del grupo que consiste de secuencias de aminoácido que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en la presente) con la secuencia de aminoácido anterior; en la cual

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 5; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores;

y/o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o solamente 1 "diferencia(s) de aminoácido" (como se define en la presente) con una de las secuencias de aminoácido anteriores, en las cuales:

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de

892

aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 5; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores;

y en las cuales:

iii) FR3 se elige del grupo que consiste de la secuencia de aminoácido:

[66] RFTISRDNAKNTVYLQMNSL**XX**EDTAVYYCAA [94] [SEC ID NO: 3]

o del grupo que consiste de secuencias de aminoácido que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en la presente) con la secuencia de aminoácido anterior; en la cual

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como

se definió en la tabla 6; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores;

y/o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o solamente 1 "diferencia(s) de aminoácido" (como se define en la presente) con una de las secuencias de aminoácido anteriores, en las cuales:

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 6; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores;

y en las cuales:

iv) FR4 se elige del grupo que consiste de la secuencia de aminoácido:

[103]**XX**QGT**X**VTVSS [113] [SEC ID NO: 4]

o del grupo que consiste de secuencias de aminoácido que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en la presente) con la secuencia de aminoácido anterior; en la cual

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 6; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores;

y/o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o solamente 1 "diferencia(s) de aminoácido" (como se define en la presente) con una de las secuencias de aminoácido anteriores, en las cuales:

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 6; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores;

y en las cuales:

v) CDR1, CDR2 y CDR3 son como se definieron anteriormente, y preferiblemente son como se definen de conformidad con las definiciones preferidas anteriores, y son más preferiblemente como se definen de conformidad con una de las definiciones más preferidas anteriores;

en las cuales los residuos distintivos se indican por "**X**" y son como se definieron anteriormente y en las cuales los números entre corchetes se refieren a las posiciones de aminoácido de conformidad con la numeración Kabat.

En otro aspecto preferido, pero no limitante, un Nanocuerpo de la invención puede tener la estructura

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

en la cual FR1 a FR4 se refieren a regiones de estructura 1 a 4, respectivamente, y en las cuales CDR1 a CDR3 se refieren a las regiones determinantes de complementariedad 1 a 3, respectivamente, y en las cuales:

i) FR1 se elige del grupo que consiste de la secuencia de aminoácido:

[1] QVQLQESGGG**L**VQAGGSLRLSCAASG [26] [SEC ID NO: 5]

o del grupo que consiste de secuencias de aminoácido que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en la presente) con la secuencia de aminoácido anterior; en la cual

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 4; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido

anteriores;

(3) el residuo distintivo en la posición es como se indicó en la secuencia anterior;

y/o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o solamente 1 "diferencia(s) de aminoácido" (como se define en la presente) con una de las secuencias de aminoácido anteriores, en las cuales:

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 4; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores; y

(3) el residuo distintivo en la posición es como se indicó en la secuencia anterior;

y en las cuales:

ii) FR2 se elige del grupo que consiste de las secuencias de aminoácido:

[36] W**F**RQAPGK**ER**E**L**VA [49] [SEC ID NO: 6]

[36] W**F**RQAPGK**ER**E**F**VA [49] [SEC ID NO: 7]

[36] W**F**RQAPGK**ER**E**G**A [49] [SEC ID NO: 8]

[36] W**F**RQAPGK**QR**E**L**VA [49] [SEC ID NO: 9]

[36] W**F**RQAPGK**QR**E**F**VA [49] [SEC ID NO: 10]

[36] W**Y**RQAPGK**GL**E**W**A [49] [SEC ID NO: 11]

o del grupo que consiste de secuencias de aminoácido que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en la presente) con una de las secuencias de aminoácido anteriores; en las cuales

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 5; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido

anteriores;

(3) los residuos distintivos en las posiciones 37, 44, 45 y 47 son como se indicaron en cada una de las secuencias anteriores;

y/o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o solamente 1 "diferencia(s) de aminoácido" (como se define en la presente) con una de las secuencias de aminoácido anteriores, en las cuales:

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 5; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores; y

(3) los residuos distintivos en las posiciones 37, 44, 45 y 47 son como se indican en cada una de las secuencias anteriores;

y en las cuales:

iii) FR3 se elige del grupo que consiste de la secuencia de

aminoácido:

[66] RFTISRDNAKNTVYLQMNSL**KP**EDTAVYYCAA [94] [SEC ID NO: 12]

o del grupo que consiste de secuencias de aminoácido que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en la presente) con la secuencia de aminoácido anterior; en la cual

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 6; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores;

(3) los residuos distintivos en las posiciones 83 y 84 son como se indican en cada una de las secuencias anteriores;

y/o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o solamente 1

"diferencia(s) de aminoácido" (como se define en la presente) con una de las secuencias de aminoácido anteriores, en las cuales:

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 6; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores; y

(3) los residuos distintivos en las posiciones 83 y 84 son como se indican en cada una de las secuencias anteriores;

y en las cuales:

iv) FR4 se elige del grupo que consiste de las secuencias de aminoácido:

[103] **WG**QGT**Q**VTVSS [113] [SEC ID NO: 13]

[103] **WG**QGT**L**VTVSS [113] [SEC ID NO: 14]

o del grupo que consiste de secuencias de aminoácido que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en la presente) con una de la secuencia de aminoácido anterior; en la cual

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 6; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores;

(3) los residuos distintivos en las posiciones 103, 104 y 108 son como se indican en cada una de las secuencias anteriores;

y/o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o solamente 1 "diferencia(s) de aminoácido" (como se define en la presente) con una de las secuencias de aminoácido anteriores, en las cuales:

903

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 6; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores; y

(3) los residuos distintivos en las posiciones 103, 104 y 108 son como se indican en cada una de las secuencias anteriores;

y en las cuales:

v) CDR1, CDR2 y CDR3 son como se definieron anteriormente, y preferiblemente son como se definen de conformidad con una de las definiciones preferidas anteriores, y son más preferiblemente como se definen de conformidad con una de las definiciones más preferidas anteriores.

En otro aspecto preferido, pero no limitante, un Nanocuerpo de la invención puede tener la estructura

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

en la cual FR1 a FR4 se refieren a regiones de estructura 1 a 4, respectivamente, y en las cuales CDR1 a CDR3 se refieren a las regiones determinantes de complementariedad 1 a 3, respectivamente, y en las cuales:

i) FR1 se elige del grupo que consiste de la secuencia de aminoácido:

[1] QVQLQESGGG**L**VQAGGSLRLSCAASG [26] [SEC ID NO: 5]

o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o solamente 1 "diferencia(s) de aminoácido" (como se define en la presente) con una de las secuencias de aminoácido anteriores, en las cuales:

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 4; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores; y

905

(3) el residuo distintivo en la posición es como se indicó en la secuencia anterior;

y en las cuales:

ii) FR2 se elige del grupo que consiste de las secuencias de aminoácido:

[36] W**F**RQAPGK**ER**E**L**VA [49] [SEC ID NO: 6]

[36] W**F**RQAPGK**ER**E**F**VA [49] [SEC ID NO: 7]

[36] W**F**RQAPGK**ER**E**G**A [49] [SEC ID NO: 8]

[36] W**F**RQAPGK**QR**E**L**VA [49] [SEC ID NO: 9]

[36] W**F**RQAPGK**QR**E**F**VA [49] [SEC ID NO: 10]

o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o solamente 1 "diferencia(s) de aminoácido" (como se define en la presente) con una de las secuencias de aminoácido anteriores, en las cuales:

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 5; y/o

(2) la secuencia de aminoácido preferiblemente

solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores; y

(3) los residuos distintivos en las posiciones 37, 44, 45 y 47 son como se indican en cada una de las secuencias anteriores;

y en las cuales:

iii) FR3 se elige del grupo que consiste de la secuencia de aminoácido:

[66] RFTISRDNAKNTVYLQMNSL**KP**EDTAVYYCAA [94] [SEC ID NO: 12]

o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o solamente 1 "diferencia(s) de aminoácido" (como se define en la presente) con una de las secuencias de aminoácido anteriores, en las cuales:

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 6; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y

ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores; y

(3) los residuos distintivos en las posiciones 83 y 84 son como se indican en cada una de las secuencias anteriores;

y en las cuales:

iv) FR4 se elige del grupo que consiste de las secuencias de aminoácido:

[103] **WG**QGT**Q**VTVSS [113] [SEC ID NO: 13]

[103] **WG**QGT**L**VTVSS [113] [SEC ID NO: 14]

o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o solamente 1 "diferencia(s) de aminoácido" (como se define en la presente) con una de las secuencias de aminoácido anteriores, en las cuales:

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como

se definió en la tabla 7; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores; y

(3) los residuos distintivos en las posiciones 103, 104 y 108 son como se indican en cada una de las secuencias anteriores;

y en las cuales:

v) CDR1, CDR2 y CDR3 son como se definieron anteriormente, y preferiblemente son como se definen de conformidad con una de las definiciones preferidas anteriores, y son más preferiblemente como se definen de conformidad con una de las definiciones más preferidas anteriores.

En otro aspecto preferido, pero no limitante, un Nanocuerpo de la invención puede tener la estructura

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

en la cual FR1 a FR4 se refieren a regiones de estructura 1 a 4, respectivamente, y en las cuales CDR1 a CDR3 se refieren a las regiones determinantes de complementariedad 1 a 3, respectivamente, y en las cuales:

i) FR1 se elige del grupo que consiste de la secuencia de aminoácido:

[1] QVQLQESGGGLVQAGGSLRLSCAASG [26] [SEC ID NO: 5]

o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o solamente 1 "diferencia(s) de aminoácido" (como se define en la presente) con una de las secuencias de aminoácido anteriores, en las cuales:

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 4; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores; y

(3) el residuo distintivo en la posición es como se indicó en la secuencia anterior;

y en las cuales:

ii) FR2 se elige del grupo que consiste de la secuencia de aminoácido:

[36] WYRQAPGKGLEWA [49] [SEC ID NO: 11]

y/o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o solamente 1 "diferencia(s) de aminoácido" (como se define en la presente) con una de las secuencias de aminoácido anteriores, en las cuales:

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 5; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores; y

(3) los residuos distintivos en las posiciones 37, 44, 45 y 47 son como se indican en cada una de las secuencias anteriores;

y en las cuales:

iii) FR3 se elige del grupo que consiste de la secuencia de aminoácido:

[66] RFTISRDNAKNTVYLQMNSL**KP**EDTAVYYCAA [94] [SEC ID NO: 12]

911

y/o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o solamente 1 "diferencia(s) de aminoácido" (como se define en la presente) con una de las secuencias de aminoácido anteriores, en las cuales:

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 6; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores; y

(3) los residuos distintivos en las posiciones 83 y 84 son como se indican en cada una de las secuencias anteriores;

y en las cuales:

iv) FR4 se elige del grupo que consiste de la secuencia de aminoácido:

[103] **WG**QGT**Q**VTVSS [113] [SEC ID NO: 13]

912

y/o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o solamente 1 "diferencia(s) de aminoácido" (como se define en la presente) con una de las secuencias de aminoácido anteriores, en las cuales:

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 7; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores; y

(3) los residuos distintivos en las posiciones 103, 104 y 108 son como se indican en cada una de las secuencias anteriores;

y en las cuales:

v) CDR1, CDR2 y CDR3 son como se definieron anteriormente, y preferiblemente son como se definen de conformidad con una de las definiciones preferidas anteriores, y son más preferiblemente como se definen de conformidad con una de las definiciones más

913

preferidas anteriores.

Algunas secuencias de estructura que pueden estar presentes en los Nanocuerpos de la invención se pueden encontrar en la patente Europea 656 946 mencionada anteriormente (véase por ejemplo también la equivalente US otorgada 5,759,808).

En otro aspecto preferido, pero no limitante, un Nanocuerpo de la invención puede tener la estructura

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

en la cual FR1 a FR4 se refieren a regiones de estructura 1 a 4, respectivamente, y en las cuales CDR1 a CDR3 se refieren a las regiones determinantes de complementariedad 1 a 3, respectivamente, y en las cuales:

i) FR1 se elige del grupo que consiste de las secuencias FR1 presentes en los Nanocuerpos de SEC ID NO's 52 a 60, SEC ID NO's 76 a 86 o SEC ID NO's 95 a 99, y en particular en los Nanocuerpos humanizados de SEC ID NO's 76 a 86 o SEC ID NO's 95 a 99,

o del grupo que consiste de secuencias de aminoácido que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en la presente) con una de las secuencias FR1; en las cuales

(1) cualquier sustitución de aminoácido en cualquier

posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 4; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con la secuencia FR1; y

(3) el residuo distintivo en la posición es como se indicó en la secuencia FR1;

y/o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o solamente 1 "diferencia(s) de aminoácido" (como se define en la presente) con una de las secuencias FR1, en las cuales:

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 4; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido,

915

comparada con la secuencia FR1; y

(3) el residuo distintivo en la posición es como se indicó en la secuencia FR1;

y en las cuales:

ii) FR2 se elige del grupo que consiste de las secuencias FR2 presentes en los Nanocuerpos de SEC ID NO's 52 a 60, SEC ID NO's 76 a 86 o SEC ID NO's 95 a 99, y en particular en los Nanocuerpos humanizados de SEC ID NO's 76 a 86 o SEC ID NO's 95 a 99,

o del grupo que consiste de secuencias de aminoácido que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en la presente) con una de las secuencias FR2; en las cuales

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 5; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con la secuencia FR2; y

916

(3) los residuos distintivos en las posiciones 37, 44, 45 y 47 son como se indican en la secuencia FR2;

y/o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o solamente 1 "diferencia(s) de aminoácido" (como se define en la presente) con una de las secuencias FR2, en las cuales:

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 5; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con la secuencia FR2; y

(3) los residuos distintivos en las posiciones 37, 44, 45 y 47 son como se indican en la secuencia FR2;

iii) FR3 se elige del grupo que consiste de las secuencias FR3 presentes en los Nanocuerpos de SEC ID NO's 52 a 60, SEC ID NO's 76 a 86 o SEC ID NO's 95 a 99, y en particular en los Nanocuerpos humanizados de SEC ID

NO's 76 a 86 o SEC ID NO's 95 a 99,

o del grupo que consiste de secuencias de aminoácido que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en la presente) con una de las secuencias FR3; en las cuales

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 6; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con la secuencia FR3; y

(3) los residuos distintivos en las posiciones 83 y 84 son como se indican en la secuencia FR3;

y/o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o solamente 1 "diferencia(s) de aminoácido" (como se define en la presente) con una de las secuencias FR3, en las cuales:

(1) cualquier sustitución de aminoácido en cualquier

posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 6; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con la secuencia FR3; y

(3) los residuos distintivos en las posiciones 83 y 84 son como se indican en la secuencia FR3;

iv) FR4 se elige del grupo que consiste de las secuencias FR4 presentes en los Nanocuerpos de SEC ID NO's 52 a 60, SEC ID NO's 76 a 86 o SEC ID NO's 95 a 99, y en particular en los Nanocuerpos humanizados de SEC ID NO's 76 a 86 o SEC ID NO's 95 a 99,

o del grupo que consiste de secuencias de aminoácido que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en la presente) con una de las secuencias FR4; en las cuales

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de

aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 6; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con la secuencia FR4; y

(3) los residuos distintivos en las posiciones 103, 104 y 108 son como se indican en la secuencia FR3;

y/o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o solamente 1 "diferencia(s) de aminoácido" (como se define en la presente) con una de las secuencias FR4, en las cuales:

(1) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva preferiblemente es ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se definió en la tabla 6; y/o

(2) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con la secuencia FR4; y

920

(3) los residuos distintivos en las posiciones 103, 104 y 108 son como se indican en la secuencia FR4;

v) CDR1, CDR2 y CDR3 son como se definieron anteriormente, y preferiblemente son como se definen de conformidad con una de las definiciones preferidas anteriores, y son más preferiblemente como se definen de conformidad con una de las definiciones más preferidas anteriores.

Algunos Nanocuerpos particularmente preferidos de la invención se pueden elegir del grupo que consiste de las secuencias de aminoácido de SEC ID NO's 52 a 60, SEC ID NO's 76 a 86 o SEC ID NO's 95 a 99, y en particular en los Nanocuerpos humanizados de SEC ID NO's 76 a 86 o SEC ID NO's 95 a 99 o del grupo que consiste de secuencias de aminoácido que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define anteriormente) con una de las secuencias de aminoácido de SEC ID NO's 52 a 60, SEC ID NO's 76 a 86 o SEC ID NO's 95 a 99; en las cuales

(1) los residuos distintivos pueden ser como se indica en la tabla 2 anterior;

(2) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva es

preferiblemente ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se define en las tablas 4-7; y/o

(3) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con las secuencias de aminoácido anteriores.

Algunos Nanocuerpos aún más particularmente preferidos de la invención se pueden elegir del grupo que consiste de las secuencias de aminoácido de SEC ID NO's 52 a 60, SEC ID NO's 76 a 86 o SEC ID NO's 95 a 99, y en particular en los Nanocuerpos humanizados de SEC ID NO's 76 a 86 o SEC ID NO's 95 a 99 o del grupo que consiste de secuencias de aminoácido que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define anteriormente) con una de las secuencias de aminoácido de SEC ID NO's 52 a 60, SEC ID NO's 76 a 86 o SEC ID NO's 95 a 99; en las cuales

(1) los residuos distintivos son como se indican en la secuencia pertinente elegida de las SEC ID NO's 52 a 60, SEC ID NO's 76 a 86 o SEC ID NO's 95 a 99;

922

(2) cualquier sustitución de aminoácido en cualquier posición diferente de una posición distintiva es preferiblemente ya sea una sustitución de aminoácido conservadora (como se define en la presente) y/o una sustitución de aminoácido como se define en las tablas 4-7; y/o

(3) la secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y ningunas supresiones o inserciones de aminoácido, comparada con la secuencia pertinente elegida de SEC ID NO's 52 a 60, SEC ID NO's 76 a 86 o SEC ID NO's 95 a 99.

Algunos de los Nanocuerpos más preferidos de la invención se pueden elegir del grupo que consiste de las secuencias de aminoácido de SEC ID NO's 52 a 60, SEC ID NO's 76 a 86 o SEC ID NO's 95 a 99, y en particular de los Nanocuerpos humanizados de SEC ID NO's 76 a 86 o SEC ID NO's 95 a 99.

Como será claro de lo anterior, el término Nanocuerpos de la invención como se usa en la presente en su sentido más amplio también comprende mutantes naturales y sintéticos, variantes, alelos, análogos y ortólogos (posteriormente colectivamente referidos como "*análogos*") de los Nanocuerpos mencionados en las SEC ID NO's 52 a 60, SEC ID NO's 76 a 86 o SEC ID NO's 95 a 99.

Generalmente, tales análogos, por ejemplo, pueden comprender secuencias homólogas, porciones funcionales, o una porción funcional de una secuencia homóloga (como se define adicionalmente posteriormente) de un Nanocuerpo. Generalmente, en tales análogos, cada residuo aminoácido (diferente del residuo distintivo) en cada una de las regiones de estructura se puede reemplazar por cualquier otro residuo aminoácido, siempre que el grado total de identidad de secuencia de las regiones de estructura permanezca como se definió anteriormente. Preferiblemente, sin embargo, en tales análogos:

- uno o más residuos aminoácido en las secuencias de estructura anteriores se reemplazan por uno o más residuos aminoácido que se presentan naturalmente en la misma posición en un dominio V_{HH} que se presenta naturalmente. Algunos ejemplos de tales sustituciones se mencionan en las tablas 4-7 anteriores;

y/o:

- uno o más residuos aminoácido en las secuencias de estructura anteriores se reemplazan por uno o más residuos aminoácido que se pueden considerar una sustitución de aminoácido "conservadora", como se describió anteriormente;

y/o:

- uno o más residuos aminoácido en las secuencias de

estructura anteriores se reemplazan por uno o más residuos aminoácido que se presentan naturalmente en la misma posición en un dominio V_H que se presenta naturalmente de un ser humano. Esto es generalmente referido como "humanización" del Nanocuerpo/V_{HH} que se presenta naturalmente en general y de la posición en particular, y será discutido con más detalle posteriormente;

y:

- las posiciones para las cuales solamente un residuo aminoácido se menciona tanto para el dominio V_H como el dominio V_{HH} en las tablas 4-7 anteriores son preferiblemente no reemplazadas.

Además, aunque generalmente es menos preferido, en tales análogos, uno o más residuos aminoácido se pueden suprimir de las regiones de estructura y/o insertan en las regiones de estructura (opcionalmente además de una o más sustituciones de aminoácido como se mencionó anteriormente), siempre que el grado total de identidad de secuencia de las regiones de estructura permanezca como se definió anteriormente. Los residuos distintivos no se deberán suprimir. Además, muy preferiblemente, los residuos aminoácido para los cuales solamente un residuo aminoácido se menciona tanto para el dominio V_H como el dominio V_{HH} en las tablas 4-7 anteriores preferiblemente no se suprimen.

925

Preferiblemente, tales análogos deberán ser tales que aún pueden enlazar, tener afinidad para y/o tener especificidad para TNF-alfa, es decir con una afinidad y/o una especificidad la cual es al menos 10%, preferiblemente al menos 50%, más preferiblemente al menos 70%, aún más preferiblemente al menos 80%, tal como al menos 90%, al menos 95%, al menos 99% o más, de la afinidad y/o especificidad de al menos uno de los Nanocuerpos de SEC ID No's 52 a 60, SEC ID NO's 76 a 86 o EC ID NO's 95 a 99, como se determina usando un ensayo adecuado, por ejemplo un ensayo para determinar el enlace del análogo a TNF, y en particular uno de los ensayos como se usan en los ejemplos posteriores.

Generalmente, tales análogos, por ejemplo, se pueden obtener proporcionando un ácido nucleico que codifica un dominio V_{HH} que se presenta naturalmente, cambiando los codones para uno o más residuos aminoácido que serán humanizados en los codones para los residuos aminoácido humanos correspondientes, expresando la secuencia de ácido nucleico/nucleótido así obtenida en un sistema de expresión u hospedador adecuado; y opcionalmente aislando y/o purificando el análogo así obtenido para proporcionar el análogo en forma esencialmente aislada (como se definió anteriormente). Esto generalmente se puede realizar usando métodos y técnicas conocidas per se, las

926

cuales serán claras para la persona experta, por ejemplo de los manuales y referencias citadas en la presente y/o de la descripción adicional posterior. Alternativamente, y por ejemplo, un ácido nucleico que codifica un análogo se puede sintetizar en una manera conocida per se (por ejemplo usando un aparato automatizado para sintetizar secuencias de ácido nucleico con una secuencia de aminoácido preferida) y se puede expresar en un sistema de expresión u hospedador adecuado, en el cual el análogo así obtenido opcionalmente se puede aislar y/o purificar para proporcionar el análogo en forma esencialmente aislada (como se definió anteriormente). Otra forma de proporciona los análogos involucra síntesis química de la secuencia de aminoácido pertinente usando técnicas para síntesis de péptido conocidas per se, tales como aquellas mencionadas posteriormente.

También generalmente será claro para la persona experta que los Nanocuerpos (incluyendo análogos de los mismos) también se pueden preparar partiendo de secuencias V_H humanas (es decir, secuencias de aminoácido o las secuencias de nucleótido correspondientes), tal como por ejemplo secuencias de V_H3 humano tales como DP-47, DP-51, DP-54 o DP-29, cambiando uno o más residuos aminoácido en la secuencia de aminoácido del dominio V_H humano, para proporcionar una secuencia de aminoácido que tiene (a) un Q

en la posición 108; y/o (b) E en la posición 44 y/o R en la posición 45, y preferiblemente E en la posición 44 y R en la posición 45; y/o (c) P, R o S en la posición 103, como se describió anteriormente. De nuevo, esto generalmente se puede realizar usando los diversos métodos y técnicas relacionadas con el párrafo previo, usando una secuencia de aminoácido y/o secuencia de nucleótido para un dominio V_H humano como un punto de partida.

El término Nanocuerpos como se usa en la presente en su sentido más amplio también comprende partes o fragmentos de los Nanocuerpos (incluyendo análogos) de la invención como se definió anteriormente, los cuales de nuevo pueden ser como se describe adicionalmente posteriormente.

Generalmente, las partes o fragmentos de los Nanocuerpos y/o análogos tienen secuencias de aminoácido en las cuales, comparadas con la secuencia de aminoácido del Nanocuerpo de longitud completa correspondiente o análogo, uno o más de los residuos aminoácido en el extremo N-terminal, uno o más residuos aminoácido en el extremo C-terminal, uno o más residuos aminoácido internos contiguos, o cualquier combinación de los mismos, se han suprimido y/o removido. También es posible combinar una o más de tales partes o fragmentos para proporcionar un Nanocuerpo de la invención.

928

Preferiblemente, la secuencia de aminoácido de un Nanocuerpo que comprende una o más partes o fragmentos de un Nanocuerpo de longitud completa y/o análogo deberá tener un grado de identidad de secuencia de al menos 50%, preferiblemente al menos 60%, más preferiblemente al menos 70%, tal como al menos 80%, al menos 90% o al menos 95%, con la secuencia de aminoácido del Nanocuerpo de longitud completa correspondiente.

Además, la secuencia de aminoácido de un Nanocuerpo que comprende una o más partes o fragmentos de un Nanocuerpo de longitud completa y/o análogo es preferiblemente tal que comprende al menos 10 residuos aminoácido contiguos, preferiblemente al menos 20 residuos aminoácido contiguos, más preferiblemente al menos 30 residuos aminoácido contiguos, tal como al menos 40 residuos aminoácido contiguos, de la secuencia de aminoácido del Nanocuerpo de longitud completa correspondiente.

Generalmente, tales partes o fragmentos de los Nanocuerpos de la invención tendrán secuencias de aminoácido en las cuales, comparadas con la secuencia de aminoácido del Nanocuerpo de longitud completa correspondiente de la invención, uno o más residuos aminoácido en el extremo N-terminal, uno o más residuos aminoácido en el extremo C-terminal, uno o más residuos

aminoácido internos contiguos, o cualquier combinación de los mismos, se han suprimido y/o removido. También es posible combinar una o más de tales partes o fragmentos para proporcionar un Nanocuerpo de la invención.

De conformidad con una modalidad preferida, un fragmento como se usa en la presente comprende al menos una de las CDR's presentes en un Nanocuerpo de tamaño completo de la invención, preferiblemente al menos dos de las CDR's presentes en un Nanocuerpo de tamaño completo de la invención, más preferiblemente al menos CDR2 y CDR3 presentes en un Nanocuerpo de tamaño completo de la invención, tal como por ejemplo todas las tres CDR's presentes en un Nanocuerpo de tamaño completo de la invención.

De conformidad con otra modalidad particularmente preferida, pero no limitante, tal parte o fragmento comprende al menos FR3, CDR3 y FR4 del Nanocuerpo de longitud completa correspondiente de la invención, es decir, como se describe, por ejemplo, en la solicitud internacional WO 03/050531 (Lasters et al.).

Preferiblemente, tales partes o fragmentos deberán ser tales que aún pueden enlazar, tener afinidad para y/o tener especificidad para TNF-alfa, es decir con una afinidad y/o una especificidad la cual es al menos 10%, preferiblemente al menos 50%, más preferiblemente al menos

930

70%, aún más preferiblemente al menos 80%, tal como al menos 90%, al menos 95%, al menos 99% o más, de la afinidad y/o especificidad del Nanocuerpo de tamaño completo correspondiente de la invención, por ejemplo un ensayo para determinar el enlace del análogo a TNF, y en particular uno de los ensayos como se usan en los ejemplos posteriores.

A partir de la descripción anterior, será claro que las secuencias de aminoácido de los Nanocuerpos usados en la presente difieren en al menos una posición de aminoácido en al menos una de las regiones de estructura de las secuencias de aminoácido de dominios V_H que se presentan naturalmente, tales como las secuencias de aminoácido de los dominios V_H que se presentan naturalmente de anticuerpos de seres humanos. En particular, será claro que las secuencias de aminoácido de los Nanocuerpos usados en la presente difieren en al menos uno de los residuos distintivos de secuencias de aminoácido de dominios V_H que se presentan naturalmente, tales como secuencia de aminoácido de dominios V_H que se presentan naturalmente de anticuerpos de Camélidos y/o seres humanos.

Por consiguiente, de conformidad con una modalidad específica, un Nanocuerpo de la invención tiene una secuencia de aminoácido que difiere en al menos una posición de aminoácido en una de las regiones de estructura de la secuencia de aminoácido de un dominio V_H que se

presenta naturalmente. De conformidad con una modalidad más específica, pero no limitante de la invención, un Nanocuerpo de la invención tiene una secuencia de aminoácido que difiere al menos uno de los residuos distintivos de la secuencia de aminoácido de un dominio V_H que se presenta naturalmente.

A partir de la descripción anterior, será claro que las secuencias de aminoácido de algunos de los Nanocuerpos de la invención, tales como Nanocuerpos humanizados de la invención, diferirán en al menos una posición de aminoácido en al menos una de las regiones de estructura (es decir, ya sea en la posición de un residuo distintivo o en otra posición) de las secuencias de aminoácido de dominios V_{HH} que se presentan naturalmente. Por consiguiente, de conformidad con una modalidad específica, pero no limitante, un Nanocuerpo de la invención tiene la secuencia de aminoácido que difiere a por lo menos en una posición de aminoácido en una de las regiones de estructura de la secuencia de aminoácidos de un dominio V_{HH} que se presenta en forma natural. De conformidad con una modalidad más específica, pero no limitativa de la invención, un Nanocuerpo de la invención tiene una secuencia de aminoácidos que difiere de al menos uno de los residuos Hallmark de la secuencia de aminoácidos de un dominio V_{HH} que se presenta de forma natural.

La invención en su sentido más amplio también comprende derivados de los Nanocuerpos de la invención. Tales derivados generalmente se pueden obtener por modificación, y en particular por modificación química y/o biológica (por ejemplo, enzimática), de los Nanocuerpos de la invención y/o de uno o más de los residuos de aminoácidos que forman los Nanocuerpos de la invención.

Los ejemplos de tales modificaciones, así como también ejemplos de residuos de aminoácidos dentro de la secuencia de Nanocuerpos que se pueden modificar de tal manera (es decir, ya sea en el esqueleto de proteína pero preferiblemente en una cadena lateral), métodos y técnicas que se pueden usar para introducir tales modificaciones y los usos potenciales y ventajas de tales modificaciones será claro para la persona experta.

Por ejemplo, una modificación puede involucrar la introducción (por ejemplo, por enlace covalente o de otra manera adecuada) de uno o más grupos funcionales, residuos o porciones en o sobre el Nanocuerpo de la invención, y en particular de uno o más grupos funcionales, residuos o porciones que confieren una o más propiedades o funcionalidades deseadas para el Nanocuerpo de la invención. Un ejemplo de tales grupos funcionales será evidente para la persona experta.

Por ejemplo, tal modificación puede comprender la

introducción (por ejemplo por enlace covalente o en cualquier otra manera adecuada) de uno o más grupos funcionales que lo que incrementa la media vida, la solubilidad y/o la absorción del Nanocuerpo de la invención, que reduce la inmunogenicidad y/o la toxicidad del Nanocuerpo de la invención, que elimina o atenúa cualesquiera efectos laterales indeseables del Nanocuerpo de la invención, y/o que confiere otras propiedades ventajosas y/o reduce las propiedades indeseadas de los Nanocuerpos y/o polipéptidos de la invención; o cualquier combinación de dos o más de los anteriores. Los ejemplos de tales grupos funcionales y de técnicas para introducirlos será claro para la persona experta, y generalmente puede comprender todos los grupos funcionales y técnicas mencionadas en la técnica anterior general citada anteriormente en la presente así como también los grupos funcionales y técnicas conocidas per se para la modificación de proteínas farmacéuticas, y en particular para la modificación de anticuerpos o fragmentos de anticuerpos (incluyendo anticuerpos de dominio único y ScFv's), para lo cual se hace referencia por ejemplo a Remington's Pharmaceutical Sciences, 16th ed., Mack Publishing Co., Easton, PA (1980). Por ejemplo, tales grupos funcionales se pueden enlazar directamente (por ejemplo covalentemente) a un Nanocuerpo de la invención, u

opcionalmente vía un enlazador o espaciador adecuado, como será evidente nuevamente para la persona experta.

Una de las técnicas más ampliamente usadas para incrementar la media vida y/o reducir la inmunogenicidad de proteínas farmacéuticas comprende unir un polímero farmacéuticamente aceptable adecuado, tal como poli(etilenglicol) (PEG) o derivados de los mismos (tal como metoxipoli(etilenglicol) o mPEG). Generalmente, se puede usar cualquier forma adecuada de pegilación, tal como la pegilación usada en la técnica para anticuerpos y fragmentos de anticuerpos (incluyendo pero sin limitarse a anticuerpos de dominio (único) y ScFv's); se hace referencia por ejemplo a Chapman, Nat. Biotechnol., 54, 531-454 (2002); por Veronese and Harris, Adv. Drug Deliv. Rev. 54, 453-456 (2003), por Harris and Chess, Nat. Rev. Drug. Discov., 2, (2003) y en WO 04/060965. Varios reactivos por pegilación de proteínas también están disponibles comercialmente, por ejemplo de Nektar Therapeutics, USA.

Preferiblemente, se usa pegilación dirigida al sitio, en particular vía un residuo de cisteína (ver por ejemplo Yang et al., Protein Engineering, 16, 10, 761-770 (2003). Por ejemplo, para este propósito, PEG se puede unir a un residuo de cisteína que se presenta en forma natural en un Nanocuerpo de la invención, un Nanocuerpo de la

invención se puede modificar para introducir adecuadamente uno o más residuos de cisteína para la unión de PEG, o una secuencia de aminoácidos que comprende uno o más residuos de cisteína para la unión de PEG se puede fusionar al N- y/o C-término de un Nanocuerpo de la invención, usando todas las técnicas de ingeniería de proteína conocidas per se para la persona experta.

Preferiblemente, para los Nanocuerpos y proteínas de la invención, un PEG se usa con un peso molecular de más de 5000, tal como más de 10,000 y menos de 200,000, tal como menos de 100,000; por ejemplo en el intervalo de 20,000-80,000.

Con respecto a la pegilación, se deberá señalar que generalmente, la invención también se incluye cualquier Nanocuerpo de la invención y/o polipéptido de la invención que ha sido pegilado en una o más posiciones de aminoácidos, preferiblemente en una forma en que la pegilación ya sea (1) incremente la media vida in vivo; (2) reduzca la inmunogenicidad; (3) proporcione una o más propiedades benéficas adicionales conocidas per se para pegilación; (4) no afecte esencialmente la afinidad del Nanocuerpo y/o polipéptido para TNF-alfa (por ejemplo, no reduce la afinidad por más de 90%, preferiblemente no más de 50%, y en forma más preferible por no más de 10%, como se determina por un ensayo adecuado, tales como aquellos

descritos en los Ejemplos posteriores); y/o (4) no afecte cualquiera de las otras propiedades deseadas de los Nanocuerpos y/o polipéptidos de la invención. Los métodos y grupos PEG adecuados para unirlos, ya sea específicamente o no específicamente, será evidente para la persona experta. Los kits y reactivos adecuados para tal pegilación se pueden obtener por ejemplo de Nektar (CA, USA).

Otros, usualmente modificaciones menos preferidas comprenden glicosilación de enlace N o de enlace O, usualmente como parte de la modificación co-traduccional y/o post-traduccional, dependiendo de la célula hospedadora usada para expresar el Nanocuerpo o polipéptido de la invención.

Todavía otra modificación puede comprender la introducción de una o más etiquetas detectables u otros grupos o porciones que generan la señal, dependiendo del uso pretendido del Nanocuerpo etiquetado. Las etiquetas y técnicas adecuadas para enlazar, usar y detectarlos será evidente para la persona experta, y por ejemplo incluye, pero no se limita a, etiquetas fluorescentes (tales como fluoresceína, isotiocianato, rodamina, ficoeritina, ficocianina, aloficocianina, o-ftaldehído, y fluorescamina y metales fluorescentes tales como ^{152}Eu u otros metales de la serie lantánido), etiquetas fosforescentes, etiquetas quimioluminiscentes o etiquetas bioluminiscentes (tales

como luminal, isoluminol, éster de acridinio teromático, imidazol, sales de acridinio, éster de oxalato, dioxetano o GFP y sus análogos), radio-isótopos (tales como ^{3}H, ^{125}I, ^{32}P, ^{35}S, ^{14}C, ^{51}Cr, ^{36}Cl, ^{57}Co, ^{58}Co, ^{59}Fe, y ^{75}Se), metales, quelatos de metales o cationes metálicos (por ejemplo cationes metálicos tales como ^{99m}Tc, ^{123}I, ^{111}In, ^{131}I, ^{97}Ru, ^{67}Cu, ^{67}Ga, y ^{68}Ga u otros metales o cationes metálicos que son particularmente adecuados para uso de diagnóstico y formación de imagen in vivo, in vitro o in situ, tales como (^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Cr, y ^{56}Fe), así como cromóforos y enzimas (tal como malato deshidrogenasa, nucleasa estafilocócica, delta-V-esteroide isomerasa, levadura de alcohol deshidrogenasa, alfa-glicerofosfato deshidrogenasa, triosa fosfato isomerasa, biotinavidin peroxidasa, peroxidasa de rábano, fosfatasa alcalina, asparaginasa, glucosa oxidasa, β-galactosidasa, ribonucleasa, ureasa, calatasa, glucosa-VI-fosfato deshidrogensas, glucoamilasa y acetilcolina esterasa). Otras etiquetas adecuadas serán claras para la persona experta y por ejemplo incluyen porciones que se pueden detectar usando espectroscopia de RMN o ESR.

Tales Nanocuerpos y polipéptidos etiquetados de la invención, por ejemplo, se pueden usar para ensayos in vitro, in vivo o in situ (incluyendo inmunoensayos per se tales como ELISA, RIA, EIA y otros "ensayos de

intercalación", etc.) así como también para propósitos de diagnóstico y formación de imagen in vivo, dependiendo de la elección de la etiqueta específica.

Como será claro para la persona experta, otra modificación puede involucrar la introducción de un grupo quelante, por ejemplo para quelar uno de los metales o cationes metálicos referidos anteriormente. Los grupos quelantes adecuados, por ejemplo incluyen, sin limitación, ácido dietilentriaminpentaacético (DTPA) o ácido etilendiamintetraacético (EDTA).

Todavía otra modificación puede comprender la introducción de un grupo funcional que es una parte de un par de enlace específico, tal como el par de enlace biotina-(estrept)avidina. Tal grupo funcional se puede usar para enlazar el Nanocuerpo de la invención a otra proteína, polipéptido o compuesto químico que se une a la otra mitad del par de enlace, es decir a través de la formación del par de enlace. Por ejemplo, un Nanocuerpo de la invención se puede conjugar a biotina, y se enlaza a otra proteína, polipéptido, compuesto o portador conjugado a avidina o estreptavidina. Por ejemplo, tal Nanocuerpo conjugado se puede usar como un reportero, por ejemplo en un sistema de diagnóstico donde un agente productor de señal detectable se conjuga a avidina o estreptavidina. Tales pares de enlace, por ejemplo, también se pueden usar para enlazar el

939

Nanocuerpo de la invención a un portador, incluyendo los portadores adecuados para propósitos farmacéuticos. Un ejemplo no limitante son las formulaciones liposomales descritas por Cao and Suresh, Journal of Drug Targetting, 8, 4, 257 (2000). Tales pares de enlace también se pueden usar para enlazar un agente terapéuticamente activo al Nanocuerpo de la invención.

Para algunas aplicaciones, en particular para aquellas aplicaciones en las cuales se propone aniquilar una célula que expresa el objetivo contra el cual los Nanocuerpos de la invención se dirigen (por ejemplo, en el tratamiento de cáncer), o reducir o alentar el crecimiento y/o proliferación tal como una célula, los nanocuerpos de la invención también se pueden enlazar a una toxina o a una porción o residuo tóxico. Los ejemplos de porciones, compuestos o residuos tóxicos los cuales se pueden enlazar a un Nanocuerpo de la invención para proporcionar - por ejemplo - un compuesto citotóxico serán claros para la persona experta y por ejemplo se pueden encontrar en la técnica previa citada anteriormente y/o en la descripción adicional en la presente. Un ejemplo es la tecnología ADEPT™ así llamada WO 03/055527.

Otras modificaciones químicas y enzimáticas potenciales serán claras para la persona experta. Tales modificaciones también se pueden introducir para propósitos

de investigación (por ejemplo, para estudiar relaciones de función-actividad). Por ejemplo, se hace referencia a Lundblad and Bradshaw, Biotechnol. Appl. Biochem., 26, 143-151 (1997).

Como se mencionó anteriormente, la invención también se refiere a proteínas o polipéptidos que comprenden al menos un dominio V_{HH} (es decir como se identifica usando los métodos de la invención) o al menos un Nanocuerpo basado en estos.

De acuerdo a una modalidad no limitante de la invención, tal polipéptido de la invención esencialmente consiste de un Nanocuerpo. Por "esencialmente consiste de" significa que la secuencia de aminoácido del polipéptido de la invención ya sea es exactamente lo mismo como la secuencia de aminoácido de un Nanocuerpo (como se menciona anteriormente) o corresponde a la secuencia de aminoácido de un Nanocuerpo, en el cual un número limitado de residuos de aminoácido, tal como 1-10 residuos de aminoácido y preferiblemente 1-6 residuos de aminoácido, tal como 1, 2, 3, 4, 5 ó 6 residuos de aminoácido, se agregan al extremo amino terminal, al extremo carboxi terminal o ambos al extremo amino terminal y al extremo carboxi terminal de la secuencia de aminoácido del Nanocuerpo.

Tales residuos de aminoácido pueden o no pueden cambiar, alterar o de otra forma influenciar las

propiedades (biológicas) del Nanocuerpo y pueden o no pueden agregar funcionalidad adicional al Nanocuerpo. Por ejemplo, tales residuos de aminoácido.

a) pueden comprender un residuo Met N-terminal, por ejemplo como resultado de expresión en una célula hospedante heteróloga u organismo hospedante.

b) puede formar una secuencia señal o secuencia líder que dirige la secreción del Nanocuerpo de una célula hospedante en la síntesis. Péptidos líderes secretorios adecuados serán claros a la persona experta, y pueden ser como se describen adicionalmente en este documento. Usualmente, tal secuencia líder estará enlazada al N-término del Nanocuerpo, a pesar que la invención está presente en su más amplio sentido no se limita a este;

c) puede formar una secuencia o señal que permite al Nanocuerpo ser dirigido hacia y/o penetrar o entrar en órganos, tejidos, células o compartimientos específicos de células, y/o que permite al Nanocuerpo penetrar o cruzar una barrera biológica tal como una membrana celular, una capa celular tal como una capa de células epiteliales, un tumor que incluye tumores sólidos, o la barrera hemato-encefálica. Ejemplos de tales secuencias de aminoácido serán claros para la persona experta. Algunos ejemplos no limitantes son los vectores de péptido pequeño ("vectores Pep-trans") descritos en el documento WO 03/026700 y en

Temsamani et al., Expert Opin. Biol. Ther., 1, 773 (2001); Temsamani y Vidal, Drug Discov. Today, 9, 1012 (004) y Rousselle, J. Pharmacol. Exp. Ther., 296, 124-131 (2001), y la secuencia translocadora de membrana descrita por Zhao et al., Apoptosis, 8, 631-637 (2003). Secuencias de aminoácido C-terminal y N-terminal para objetivo intracelular de fragmentos de anticuerpo son por ejemplo, descritos por Cardinales et al., Methods, 34, 171 (2004). Otras técnicas adecuadas para objetivo intracelular involucran la expresión y/o uso de los así llamados "anticuerpos" que comprenden un Nanocuerpo de la invención, como se menciona abajo;

d) puede formar una "etiqueta", por ejemplo una secuencia o residuo de aminoácido que permite o facilita la purificación del Nanocuerpo, por ejemplo, usando técnicas de afinidad dirigidas contra la secuencia o residuo. En adelante, la secuencia o residuo puede ser removido (por ejemplo, por desdoblamiento químico o enzimático) para proporcionar la secuencia de Nanocuerpo (para este propósito, la etiqueta puede opcionalmente estar enlazada a la secuencia de Nanocuerpo vía una secuencia enlazadora desdoblada o contiene una porción desdoblada). Algo preferido, pero ejemplos no limitantes de tales residuos son residuos de histidina múltiple, residuos de glutaniona y una etiqueta myc tal como AAAEQKLISEEDLNGAA [SEC ID NO.

476];

e) puede ser uno o más residuos de aminoácido que han sido funcionalizados y/o que pueden servir como un sitio para unión de grupos funcionales. Residuos de aminoácido adecuados y grupos funcionales serán claros a la persona experta e incluye, pero no se limita a, los residuos de aminoácido y grupos funcionales mencionados en este documento por los derivados de los Nanocuerpos de la invención.

De acuerdo a otra modalidad, un polipéptido de la invención comprende un Nanocuerpo de la invención, el cual se fusiona a su extremo amino terminal, a su extremo carboxi terminal, o ambos a su extremo amino terminal y a su extremo carboxi terminal por al menos una secuencia de aminoácido adicional, es decir, para proporcionar una fusión de proteína, que comprende el Nanocuerpo de la invención y a una o más de las secuencias de aminoácido adicional. Tal fusión también será referida en este documento como una "fusión de Nanocuerpo".

Una o más de las secuencias de aminoácido adicional puede ser cualquiera de las secuencias de amino adecuada y/o deseada. Las secuencias de aminoácido adicional pueden o no pueden cambiar, alterar o de otra forma influenciar las propiedades (biológicas) del Nanocuerpo, y puede o no puede agregar funcionalidad

adicional al Nanocuerpo o el polipéptido de la invención. Preferiblemente, la secuencia de aminoácido adicional es de forma tal que confiere una o más propiedades deseadas o funcionalidades al Nanocuerpo o el polipéptido de la invención.

Ejemplos de tales secuencias de aminoácido serán claros a la persona experta, y pueden generalmente comprender todas las secuencias de aminoácido que se usan en fusiones del péptido en base a anticuerpos convencionales y fragmentos del mismo (que incluyen, pero no se limitan a anticuerpos de dominio único o ScFv'). Se hace referencia por ejemplo a la revisión por Holliger y Hudson, Nature Biotechnology, 23, 9, 1126-1136 (2005).

Por ejemplo, tal secuencia de aminoácido puede ser una secuencia de aminoácido que incrementa la vida media, la solubilidad o la absorción, reduce la inmunogenicidad o la toxicidad, elimina o atenúa los efectos colaterales indeseables, y/o confiere otras propiedades ventajosas a y/o reduce las propiedades indeseadas de los polipéptidos de la invención, comparado al Nanocuerpo de la invención per se. Algunos ejemplos no limitantes de tales secuencias de aminoácido son proteínas de suero, tal como albúmina de suero humano (véase por ejemplo, documento WO 00/27435) o moléculas hapténicas (por ejemplo, haptenos que son reconocidos por anticuerpos que

circulan, véase por ejemplo el documento WO 98/22141).

La secuencia de aminoácido adicional puede también proporciona un segundo sitio enlazante, dicho sitio enlazante puede ser dirigido contra cualquier proteína deseada, polipéptido, antígeno, determinante antigénico o epítope (que incluye pero no se limita a la misma proteína, polipéptido, antígeno, determinante antigénico o epítope contra el cual en Nanocuerpo de la invención se dirige, o una proteína diferente, polipéptido, antígeno, determinante antigénico o epítope). Por ejemplo, la secuencia de aminoácido adicional puede proporciona un segundo sitio enlazante que se dirige contra una proteína de suero (tal como, por ejemplo, albúmina de suero humano u otra proteína de suero tal como IgG), para proporcionar vida media incrementada en suero. Se hace referencia por ejemplo, a los documento EP 0 368 684, WO 91/01743, WO 01/45746 y WO 04/003019 (en los cuales se mencionan varias proteínas de suero), la solicitud internacional por el solicitante titulada "Nanobodies TM contra beta amiloide y polipéptidos que comprenden los mismos para el tratamiento de enfermedades neural degenerativas tal como enfermedad de Alzheimer" (en la cual se mencionan otras proteínas variadas), así como por Harmsen et al., Vaccine, 23 (41); 4926-42.

De acuerdo a otra modalidad, una o más secuencias

de aminoácido adicional pueden comprender una o más partes, fragmentos o dominios de anticuerpos de cadena 4 convencional (y en anticuerpos humanos particulares) y/o de anticuerpos de cadena pesada. Por ejemplo, aunque usualmente menos preferido, un Nanocuerpo de la invención puede estar enlazada a un dominio V_H o V_L (preferiblemente humano) o para un análogo natural o sintético de un dominio V_H o V_L, nuevamente opcionalmente vía una secuencia enlazadora (que incluye pero no se limita a otros anticuerpos de dominio (único), tal como los dAb descritos por Ward et al.).

Al menos un Nanocuerpo también puede ser enlazado a uno o más dominio CH_1, CH_2 y/o CH_3 (preferiblemente humano), opcionalmente vía una secuencia enlazadora. Por ejemplo, un Nanocuerpo enlazado a un dominio CH_1 adecuado, puede por ejemplo, ser usado junto con cadenas ligeras adecuadas, para generar análogos de fragmentos/estructuras de anticuerpo para fragmentos Fab o fragmentos F(ab')2 convencional, pero en el cual uno o (en caso de un fragmento F(ab')2) uno o ambos de los dominios V_H convencional pueden ser reemplazados por un Nanobody de la invención. También, dos Nanocuerpos pueden ser enlazados a un dominio CH3 (opcionalmente vía un enlazador) para proporcionar un constructo con vida media incrementada in vivo.

De acuerdo a una modalidad específica de la invención, uno o más Nanocuerpos de la invención pueden enlazarse a una o más partes, fragmentos o dominios del anticuerpo que confiere una o más funciones efectoras al polipéptido de la invención y/o puede conferir la capacidad para unirse a uno o más receptores Fc. Por ejemplo, para este propósito, y sin ser limitado a esto, una o más secuencias de aminoácido adicional pueden comprender uno o más dominio CH_2 y/o CH_3 de un anticuerpo, tal como de un anticuerpo de cadena pesada (como se describe en este documento) y más preferiblemente de un anticuerpo de cadena 4 humana convencional; y/o puede formar (parte de) y región Fc, por ejemplo de IgG, de IgE o de otro Ig humano. Por ejemplo, el documento WO 94/04678 describe anticuerpos de cadena pesada que comprende un dominio V_{HH} Camélido o un derivado del mismo humanizado (es decir, un Nanocuerpo), en el cual, el dominio CH_2 y CH_3 Camelidae pueden ser reemplazados por dominio CH_2 y CH_3 humanos, para proporcionar una inmunoglobulina que contiene 2 cadenas pesadas, cada una comprende un Nanocuerpo y dominio CH2 y CH3 (pero no dominio CH1), dicha inmunoglobulina tiene la función efectora proporcionada por los dominios CH2 y CH3 y dicha inmunoglobulina puede funcionar sin la presencia de cualquier cadena ligera. Otras secuencias de aminoácido que puede ser adecuadamente enlazado a los Nanocuerpos de la

invención para proporcionar una función efectora, será claro a la persona experta, y puede ser escogida en las bases de las funciones afectoras deseadas. Se hace referencia por ejemplo a los documentos WO 04/058820, WO 99/42077 y WO 05/017148, así como a la revisión por Hollinger y Hudson, supra. El acoplamiento de un Nanocuerpo de la invención a una porción Fc también se puede conducir a una vida media incrementada, comparada al Nanocuerpo correspondiente de la invención. Para algunas aplicaciones, el uso de una porción Fc y/o de dominios constantes (es decir, dominios CH_2 y/o CH_3) que confiere vida media incrementada sin cualquier función efectora biológicamente significante puede también ser adecuada o aún preferida. Otros constructos adecuados que comprenden uno o más Nanocuerpos y uno o más dominios constantes con vida media incrementada in vivo, será claro para una persona experta, y puede por ejemplo comprender dos Nanocuerpos enlazados a un dominio CH3, opcionalmente vía una secuencia enlazadora. Generalmente, cualquier proteína o derivado de fusión con vida media incrementada preferiblemente tendrá un peso molecular de más de 50 kD, el valor corte de para absorción renal.

Las secuencias de aminoácido adicional también pueden formar una secuencia señal o una secuencia líder que dirige la secreción del Nanocuerpo o el polipéptido de la

invención de una célula hospedante en la síntesis (por ejemplo para proporcionar una pre-, pro- o prepo-forma del polipéptido de la invención, dependiendo de la célula hospedante usada para expresar el polipéptido de la invención).

La secuencia de aminoácido adicional puede también formar una secuencia o señal que permite al Nanocuerpo o polipéptido de la invención para ser dirigido hacia y/o para penetrar o entrar en órganos, tejidos, células o partes o compartimientos específicos de células, y/o que permite al Nanocuerpo o polipéptido de la invención para penetrar o atravesar una barrera biológica, tal como membrana celular, una capa celular tal como una capa de células epiteliales, un tumor que incluye tumores sólidos, o la barrera hemato-encefálica. Ejemplos adecuados de tales secuencias de aminoácido serán claros para una persona experta, y por ejemplo incluye, pero no se limita a, los vectores "Peptrans" mencionados anteriormente, las secuencias descritas por Cardinale et al., y las secuencias de aminoácido y los fragmentos de anticuerpo conocidos per se, que se pueden usar para expresar o producir los Nanocuerpos y polipéptidos de la invención también llamados "intracuerpos", por ejemplo, como se describe en los documento WO 94/02610, WO 95/22618, US-A-7004940, WO 03/014960, WO 99/07414; WO 05/01690; EP 1 512 696; y en

Cattaneo, A. & Biocca, S. (1997) Intracellular Antibodies: Development and Applications. Landes and Springer-Verlag; y en Kontermann, Methods 34, (2004) 163-170, y las referencias adicionales descritas en este documento.

Para algunas aplicaciones, en particular para aquellas aplicaciones en las cuales se pretende eliminar una célula que expresa el objetivo contra el cual los Nanocuerpos de la invención se dirigen (por ejemplo, en el tratamiento de cáncer), o para reducir o disminuir el crecimiento y/o proliferación de tal célula, los Nanocuerpos de la invención pueden también estar enlazados a un Nanocuerpo de la invención, para proporcionar, por ejemplo, un polipéptido citotóxico de la invención, será claro para una persona experta y puede por ejemplo ser encontrado en la técnica anterior citada anteriormente y/o en la descripción adicional en este documento. Un ejemplo es la así llamada tecnología ADEPT™ del documento WO 03/055527.

De acuerdo a una modalidad no limitante, se pueden agregar uno o más residuos de aminoácido a, insertar en y/o sustituir en la secuencia de aminoácido de un Nanocuerpo o polipéptido de la invención, para proporcionar uno o más residuos de aminoácido específico para unirse a un grupo PEG.

La eficacia de farmacéuticos de proteína

dependiente en su potencia para neutralizar el objetivo, pero también en los farmacocinéticos intrínsecos del potencial del fármaco. Debido a que los riñones generalmente filtran moléculas abajo de 60,000 Daltons (Da), se han enfocado esfuerzos para reducir el espacio libre en el incremento de peso molecular del biofarmacéutico a través de fusiones de proteínas (Syred et al., 1997), glicosilaciones o modificación con polímeros de polietilen glicol, es decir, PEGilación (Lee et al., 1999; Abuchowski et al., 1977; Nucci et al., 1991; Lecolley, et al. Chem Commun, 2004; Tao et al., J Am Chem Soc, 2004; Mantovani et al., 2005). Estos métodos satisfactoriamente prolongan la exposición in vivo del biofarmacéutico.

Alternativamente, la vida media se puede prolongar usando otro agente de pegilación, POLI PEG para conjugar a los Nanocuerpos bivalentes, TNF56 o TNF55. Los POLI PEG son polímeros de forma apanalada con dientes PEG en una estructura metacrílica. Los POLI PEGS pueden variar en longitud de la cadena PEG, en una estructura metacrílica y en el grupo final activo, el cual determina el método de conjugación del POLI PEG para el Nanocuerpo. La conjugación del sitio específico a la cisteína C-terminal presente en los Nanocuerpos se puede lograr a través del grupo final de maleimida activo en el POLI PEG.

La invención también abarca cualquier Nanocuerpo

de la invención y/o polipéptido de la invención que se ha glicosilado en una o más posiciones de aminoácido, usualmente dependiendo del hospedante usando para expresar el Nanocuerpo o polipéptido de la invención (como se describe adicionalmente abajo).

De acuerdo a una modalidad no limitante, uno o más residuos de aminoácido se pueden agregar a, insertar en y/o sustituir en la secuencia de aminoácido de un Nanocuerpo o polipéptido de la invención, para proporcionar uno o más residuos de aminoácido específico y/o un sitio que puede ser glicosilado por el organismo hospedante usando. Por medio de un ejemplo preferido, pero no limitante, el residuo N en posición 50 con CDR2 de un Nanocuerpo de la invención puede por ejemplo ser reemplazado por un residuo Q, D o S para proporcionar un sitio de glicosilación, por ejemplo, para glicosilación por *Pichia*.

De acuerdo a otra modalidad, un polipéptido de la invención puede comprender la secuencia de aminoácido de un Nanocuerpo, el cual se fusiona en su extremo amino terminal, en su extremo carboxi terminal o ambos en su extremo amino terminal y en su extremo carboxi terminal con al menos una secuencia de aminoácido adicional.

Nuevamente, las secuencias de aminoácido adicional pueden o no pueden cambiar, alterar o de otra

forma influenciar las propiedades (biológicas) del Nanocuerpo y puede o no puede agregar funcionalidad adicional al Nanocuerpo.

Por ejemplo, de acuerdo a una modalidad preferida, pero no limitante, la secuencia de aminoácido adicional puede comprender al menos un Nanocuerpo adicional, para proporcionar un polipéptido de la invención que comprende al menos dos, tal como tres, cuatro o cinco Nanocuerpos, en los cuales los Nanocuerpos pueden opcionalmente ser enlazados vía una o más secuencias enlazadoras (como se define en este documento).

Los polipéptidos de la invención que comprenden dos o más Nanocuerpos también se referirán en este documento como polipéptidos "multivalentes". Por ejemplo un polipéptido "bivalente" de la invención, comprende dos Nanocuerpos, opcionalmente enlazados vía una secuencia enlazadora, mientras un polipéptido "trivalente" de la invención comprende tres Nanocuerpos, opcionalmente enlazado vía dos secuencias enlazadoras; etc.

En un polipéptido multivalente de la invención, los dos o más Nanocuerpos pueden ser los mismos o diferentes. Por ejemplo, los dos o más Nanocuerpos en un polipéptido multivalente de la invención:

- se puede dirigir contra el mismo antígeno, es decir, contra las mismas partes o epítopes del antígeno o

contra dos o más partes o epítopes diferentes del antígeno; y/o;

- se puede dirigir contra los antígenos diferentes;

o una combinación de los mismos.

Así, un polipéptido de la invención, por ejemplo:

- puede comprender dos Nanocuerpos idénticos;

- puede comprender un primer Nanocuerpo dirigido contra una primera parte o epítope de un antígeno y un segundo Nanocuerpo dirigido contra la misma parte o epítope del antígeno o contra otra parte o epítope del antígeno;

- o puede comprender un primer Nanocuerpo dirigido contra un primer antígeno y un segundo Nanocuerpo dirigido contra un segundo antígeno diferente del primer antígeno;

mientras un Polipéptido trivalente de la invención, por ejemplo:

- puede comprender tres Nanocuerpos idénticos o diferentes dirigidos contra las partes o epítopes iguales o diferentes del mismo antígeno;

- puede comprender dos Nanocuerpos idénticos o diferentes dirigidos contra las partes o epítopes iguales o diferentes en un primer antígeno y un tercer Nanocuerpo dirigido contra un segundo antígeno diferente del primer antígeno; o

- puede comprender un primer Nanocuerpo dirigido contra un primer antígeno, un segundo Nanocuerpo dirigido contra un segundo antígeno diferente del primer antígeno, y un tercer Nanocuerpo dirigido contra un tercer antígeno del primero y segundo antígeno, polipéptidos de la invención que contienen al menos dos Nanocuerpos, en el cual al menos un Nanocuerpo se dirige contra un primer antígeno y al menos un Nanocuerpo se dirige contra un segundo Nanocuerpo diferente del primer antígeno, también se referirá como Nanocuerpos "multiespecíficos". Así, un Nanocuerpo "biespecífico" es un Nanocuerpo que comprende al menos un Nanocuerpo dirigido contra un primer antígeno y al menos un Nanocuerpo adicional dirigido contra un segundo antígeno, mientras un Nanocuerpo "triespecífico" es un Nanocuerpo que comprende al menos un Nanocuerpo dirigido contra un primer antígeno, al menos un Nanocuerpo adicional dirigido contra un segundo antígeno, y al menos un Nanocuerpo adicional dirigido contra un tercer antígeno; etc.

Por lo tanto, en su forma más simple, un polipéptido biespecífico de la invención es un polipéptido bivalente de la invención (como se define en este documento), que comprende un primer Nanocuerpo dirigido contra un primer antígeno y un segundo Nanocuerpo dirigido contra un segundo antígeno, en el cual dicho primero y segundo Nanocuerpo puede opcionalmente estar enlazado vía

una secuencia enlazadora (como se define en este documento); en donde un polipéptido triespecífico de la invención en su forma más simple es un polipéptido trivalente de la invención (como se define en este documento), que comprende un primer Nanocuerpo dirigido contra un primer antígeno, un segundo Nanocuerpo se dirige contra un segundo antígeno y un tercer Nanocuerpo dirigido contra un tercer antígeno, en el cual el primero, segundo y tercer Nanocuerpo puede opcionalmente estar enlazado vía uno o más, y en particular uno o más en dos secuencias enlazadoras particulares.

Sin embargo, como será claro de la descripción anteriormente, la invención no se limita a esto, en el sentido que un polipéptido multiespecífico de la invención puede comprender cualquier número de Nanocuerpos dirigidos contra dos o más antígenos diferentes.

Para polipéptidos multivalente y multiespecífico que contiene uno o más dominios V_{HH} y su preparación, también se hace referencia a Conrath et al., J. Biol. Chem., vol. 276, 10. 7346-7350, así como al documento EP 0 822 985.

En los polipéptidos de la invención, uno o más Nanocuerpos y uno o más polipéptidos pueden ser directamente enlazados entre sí (como por ejemplo, se describe en el documento WO 99/23221) y/o puedes estar

enlazados entre sí vía uno o más espaciadores o enlazadores adecuados o cualquier combinación de los mismos.

Espaciadores o enlazadores adecuados para uso en polipéptidos multivalentes o multiespecíficos serán claros a una persona experta, y puede generalmente ser cualquier enlazador o espaciador usado en la técnica para enlazar secuencias de aminoácido. Preferiblemente, el enlazador o espaciador es adecuado para uso en proteínas constructo o polipéptidos que se pretenden para uso farmacéutico.

Algunos espaciadores particularmente preferidos incluyen los espaciadores y enlazadores que se usan en la técnica para enlazar fragmentos de anticuerpo o dominios de anticuerpo. Estos incluyen los enlazadores mencionados en el antecedente general de la técnica citado anteriormente, así como por ejemplo, enlazadores que se usan en la técnica para anticuerpos constructo o fragmentos ScFv (en este aspecto, sin embargo, se notará que mientras en anticuerpos y en fragmentos ScFv, la secuencia enlazadora usada debe tener una longitud, un grado de flexibilidad u otras propiedades que permite a los dominios V_H y V_L pertinentes llegar juntos para formar el sitio enlazante al antígeno completo, no hay limitación particular en la longitud o la flexibilidad del enlazador usado en el polipéptido de la invención, ya que cada Nanocuerpo por si mismo forma un sitio enlazante al antígeno completo).

Otros enlazadores adecuados generalmente comprenden compuestos orgánicos o polímeros, en particular aquellos adecuados para uso en proteínas para uso farmacéutico. Por ejemplo, porciones poli(etilenglicol) se han usado para enlazar dominios de anticuerpo, véase por ejemplo el documento WO 04/081026.

También dentro del alcance de la invención, los enlazadores usados confieren uno o más de otras propiedades favorables o funcionalidad a los polipéptidos de la invención, y/o proporciona uno o más sitios para la formación de derivados y/o para la unión de grupos funcionales (por ejemplo, como se describe en este documento para los derivados de los Nanocuerpos de la invención). Por ejemplo, enlazadores que contienen uno o más residuos aminoácidos cargados (véase Tabla A-3 anteriormente) puede proporcionar propiedades hidrofílicas mejoradas, mientras enlazadores que forman o contienen epítopes o etiquetas pequeñas se pueden usar para los propósitos de detección, identificación y/o purificación. Nuevamente, en base a la descripción en este documento, la persona experta será capaz para determinar los enlazadores óptimos para uso en un polipéptido específico de la invención, opcionalmente después de algunos experimentos de rutina limitados.

Finalmente, cuando do o más enlazadores se usan

en los polipéptidos de la invención, estos enlazadores pueden ser el mismo o diferente. Nuevamente, en base a la descripción en este documento, la persona experta será capaz para determinar los enlazadores óptimos para uso en un polipéptido específico de la invención, opcionalmente después de algunos experimentos de rutina limitados.

Enlazadores para uso en polipéptidos multivalentes y multiespecíficos serán claros a la persona experta, y por ejemplo incluye enlazadores gly-ser, por ejemplo del tipo $(gly_xser_y)_z$, tal como (por ejemplo $(gly_4ser)_3$ o $(gly_3ser_2)_3$, como se describe en el documento WO 99/42077, regiones similares a articulaciones tales como las regiones de articulación de anticuerpos de cadena pesada que se originan naturalmente o secuencias similares. Para otros enlazadores adecuados, también se hace referencia al antecedente general de la técnica citado anteriormente. Algunos enlazadores particularmente preferidos se dan en las SEC ID NOs. 68 y 69.

Enlazadores también pueden proporcionar alguna funcionalidad para el polipéptido multivalente o multiespecífico. Por ejemplo, enlazadores que contienen uno o más residuos de aminoácido cargado (véase Tabla 1 anteriormente) puede proporcionar propiedades hidrofílicas mejoradas, mientras enlazadores que forman o contienen epítopes o etiquetas pequeñas se pueden usar para los

propósitos de detección, identificación y/o purificación.

Como se menciona en este documento, en una proteína o polipéptido de la invención, los Nanocuerpos anti-TNF mencionados en este documento son preferiblemente enlazados en tal forma que la proteína o pilipéptido, en enlace a un trímero THF, es capaz de inhibir o reducir el receptor TNF reticulado que es mediado por el trímero TNF y/o la transducción de señal que es mediada por el receptor reticulado; y/o en tal forma que la proteína o polipéptido es capaz de enlazarse intramolecularmente a al menos dos sitios enlazados al receptor THF en un trímero TNF. Enlazadores adecuados son como se describen en este documento.

Como también se menciona en este documento, si una proteína o polipéptido proporciona enlace intermolecular o enlace extramolecular se pude valorar (al menos inicialmente) por una cromatografía de exclusión de tamaño. Por Cromatografía de Exclusión de Tamaño, los complejos de TNF alfa y anticuerpos pueden ser analizados para determinar el número y la proporción de anticuerpo y moléculas TNF alfa en el complejo. A partir de estos datos, se puede deducir si se originan enlaces inter- o intramoleculares, como se hizo por Santora y colegas (Santora, L.C., et al., Anal Biochem. 2001) para estabiliza la estequiometria de enlace del anticuerpo D2E7 monoclonal

(Humira) para TNF alfa a proporciones diferentes de anticuerpo y etiqueta. A partir del peso molecular del complejo se concluye que tres moléculas de anticuerpo sometidas a complejo con tres trímeros TNF, con ello indicando que los enlaces de anticuerpo en una forma intermolecular. Experimentos similares se realizan con Nanocuerpos bivalentes, en los cuales un enlazador muy corto induce la formación de complejos moleculares grandes, los cuales se obtienen por uniones intermoleculares. Sin embargo, algunos constructos de Nanocuerpos bivalentes con enlazadores más largos eluidos de la columna de filtración en gel como complejos pequeños discretos, con ello se demuestra que se forman las uniones intramoleculares. Combinados con los datos de bioensayo, en los cuales en enlazador más largo que contiene Nanocuerpo TNF1 tiene una potencia óptima (neutralización completa de cantidad de TNF usada en el ensayo, es decir, 10 pM), se puede concluir que en enlace intramolecular del Nanocuerpo bivalente, efectivamente previene la reticulación de dos receptores unidos a la célula y la activación del receptor asociado. Anticuerpos monoclonales conocidos tales como Humira o Remicade pueden no formar tales uniones intramoleculares, que conduce siempre dos sitios de enlace al receptor en la molécula TNF trimérica para un cierto grado disponible para interacción con el receptor unido a la célula, el cual se

traduce en neutralización menos potente como se mide en el bioensayo.

Alternativamente, si una proteína o polipéptido proporciona enlace intermolecular o enlace extramolecular puede ser valorada por cristalografía y/o modelado molecular (u otro adecuado en técnicas de sílice). Un modelo de un complejo TNF30/TNF alfa trimérico se genera en base a la estructura de cristal del complejo TNF1/TNF alfa de tipo nativo, monomérico. Para esta estructura, se modela el constructo enlazador TNF30-enlazador ALB8-TNF30 final. Se modela el constructo enlazador TNF30-enlazador ALB8-TNF30 partiendo del trímero de TNFα con dos moléculas TNF30 unidas. Como la estructura del ALB8 no se conoce, en lugar se usa una tercera molécula TNF30, la cual se coloca entre los otros dos Nanocuerpos a lo largo de la línea, entre el N- y C-termino. Los enlazadores de 9 aminoácidos, después se agregan manualmente.

El modelo se muestra en la Figura 62. Claramente, los enlazadores de 9 aminoácidos junto con la ALB8, proporciona suficiente espacio para abarcar aproximadamente 66 Å entre los dos dominios TNF30 unidos a TNFα. ALB8 por si misma abarca 40 Å, y cada enlazador puede abarcar otro ~27 Å en conformación completamente extendida. Como un resultado, la ALB8 tiene completamente algo de flexibilidad de movimiento, y no se espera que se enlace a albúmina,

puede interferir mucho con en enlace a TNFα.

Sin embargo, es igualmente que los enlazadores pueden ser acortados sin afectar la avidez, especialmente en el caso den enlazador que es C-terminal de ALB8. Esto puede tener el efecto benéfico de incrementar en el enlace al mismo trímero TNFα contra los trímeros reticulados, porque la probabilidad que el segundo TNF30 asociado con un diferente TNFα incrementado con la longitud del enlazador.

Como también se describe adicionalmente en este documento, un polipéptido multiespecífico de la invención dirigido contra un antígeno deseado y contra al menos una proteína de suero, tal como las proteínas de suero mencionadas posteriormente en este documento, y en particular contra albúmina de suero humano, puede mostrar vida media incrementada en suero, comparado al Nanocuerpo monovalente correspondiente.

Como se menciona en este documento anteriormente, los métodos descritos en este documento son particularmente adecuados para generar tal multivalente de polipéptidos multiespecíficos de la invención.

En un polipéptido de la invención, al menos un Nanocuerpo también puede estar enlazado a un dominio VH convencional o para un análogo natural o sintético de un dominio V_H, opcionalmente vía una secuencia enlazadora.

En un polipéptido de la invención, al menos un

Nanocuerpo puede también estar enlazado a un dominio V_L o para un análogo natural o sintético de un dominio V_L, opcionalmente vía una secuencia enlazadora, para proporcionar un polipéptido de la invención que está en la forma análoga a un fragmento scFv convencional, pero que contiene un Nanocuerpo en lugar de un dominio V_H.

En un polipéptido de la invención, al menos un Nanocuerpo puede también estar enlazado a uno o más de un dominio CH_1, CH_2 y/o CH_3, opcionalmente vía una secuencia enlazadora. Por ejemplo, un Nanocuerpo enlazado a un dominio CH_1 adecuado, puede por ejemplo, ser usado junto con cadenas ligeras adecuadas, para generar análogos de fragmento/estructuras de anticuerpo para fragmentos Fab convencional o fragmentos $F(ab')_2$, pero en el cual uno o (en caso de un fragmento $F(ab')_2$) uno o ambos de los dominios VH convencionales pueden ser reemplazados por un Nanocuerpo. Tales fragmentos también pueden ser heteroespecíficos o biespecíficos, es decir, dirigido contra dos o más antígenos. Un Nanocuerpo enlazado a dominios CH_2 y CH_3 adecuados, por ejemplo derivados de Camélidos, pueden ser usados para formar un anticuerpo de cadena pesada nanoespecífica o biespecífica. Finalmente, un Nanocuerpos enlazado a dominios CH1, CH2 y CH3, por ejemplo derivado de un humano, debe ser usado, junto con cadenas ligeras adecuadas, para formar un anticuerpo que es análogo

a un anticuerpo de 4 cadenas convencional, pero en el cual uno o ambos de los dominios V_H convencional se han reemplazado por un Nanocuerpo.

También, además a uno o más Nanocuerpos, polipéptidos de la invención pueden también contener grupos, porciones o residuos funcionales, por ejemplo, sustancias terapéuticamente activas, tal como aquellos mencionados posteriormente, y/o marcadores y marcas, tal como marcadores fluorescentes, isotopos, etc., como se describe adicionalmente posteriormente en este documento.

Los Nanocuerpos de la invención, los polipéptidos de la invención, y ácidos nucleicos que codifican el mismo, pueden ser preparados en una manera conocida per se, como será a una persona experta a partir de la descripción adicional en este documento. Algunos métodos preferidos, pero no limitantes para preparar los Nanocuerpos, polipéptidos y ácidos nucleicos incluyen los métodos y técnicas mencionadas anteriormente y/o descritas adicionalmente en este documento posteriormente.

Como será claro a una persona experta, un método particularmente útil para preparar un Nanocuerpo y/o un polipéptido de la invención, generalmente comprende las etapas de:

- la expresión, en una célula hospedante u organismo hospedante adecuado (también referido en este

documento como un "*hospedante de la invención*") o en otro sistema de expresión adecuado de un ácido nucleico que codifica el Nanocuerpo o polipéptido de la invención (también referido en este documento como un "*ácido nucleico de la invención*"), opcionalmente seguido por:

- aislar y/o purificar el nanocuerpo o polipéptido de la invención, así obtenido.

En particular, tal método puede comprender las etapas de:

- cultivar y/o mantener un hospedante de la invención bajo condiciones que son de tal forma que el hospedante de la invención expresa y/o produce al menos un Nanocuerpo y/o polipéptido de la invención; opcionalmente seguido por:

- aislar y/o purificar el Nanocuerpo o polipéptido de la invención así obtenido.

Un ácido nucleico de la invención puede estar en la forma de ADN o ARN ramificada única o doble, y es preferiblemente en la forma de ADN de ramificado doble. Por ejemplo, las secuencias del nucleótido de la invención puede ser ADN genómico, ADNc, ADN sintético (tal como ADN con un codón utilizado que ha sido específicamente adaptado para expresión en la célula hospedante u organismo hospedante planeado).

De acuerdo a una modalidad de la invención, el

ácido nucleico de la invención es esencialmente asilado, como se define en este documento anteriormente.

El ácido nucleico de la invención también está en la forma de, estar presente en y/o ser parte de un vector, tal como por ejemplo un plásmido, cósmido o YAC, el cual nuevamente puede estar en forma esencialmente aislada.

Los ácidos nucleicos de la invención, pueden ser preparados u obtenidos en una manera conocida per se, en base a la información en las secuencias de aminoácido para los polipéptidos de la invención, proporcionados en este documento, y/o pueden ser aislados de una fuente natural adecuada. Para proporcionar análogos, secuencias de nucleótido que codifican dominios V_{HH} que se originan de forma natural, pueden por ejemplo ser sometidos a mutagénesis dirigida al sitio, para proporcionar un ácido nucleico de la invención que codifica en análogo. También, será claro a la persona experta, preparar un ácido nucleico de la invención, también varias secuencias de nucleótido, tal como al menos una secuencia de nucleótido que codifica un Nanocuerpo y por ejemplo ácidos nucleicos que codifican uno o más enlazadores, pueden estar enlazados juntos en una manera adecuada.

Técnicas para generar los ácidos nucleicos de la invención, serán claras a la persona experta y puede por ejemplo incluir, pero no se limita a, síntesis de ADN

automatizada; mutagénesis dirigida al sitio; combinar dos o más secuencias sintéticas o que se origina de forma natural (o dos o más partes de la misma), introducción de mutaciones que conduce a la expresión de un producto de expresión truncado; introducción de uno o más sitios de restricción (por ejemplo, para crear casetes y/o regiones que pueden fácilmente ser digeridos y/o ligados usando enzimas de restricción adecuadas), y/o la introducción de mutaciones por medios de una reacción RCP usando uno o más cebadores "apareados de manera desigual", usando por ejemplo, una secuencia de un GPCR que se origina de forma natural como una plantilla. Estas y otras técnicas serán claras a la persona experta, y nuevamente se hace referencia a los manuales, tal como Sambrook et al., y Ausubel et al., mencionado anteriormente, así como en los Ejemplos de abajo.

El ácido nucleico de la invención puede también estar en la forma de, ser presente en y/o ser parte de un constructo genético, será claro a una persona experta en la técnica. Tales constructos genéticos generalmente comprenden al menos un ácido nucleico de la invención, que es opcionalmente enlazada a uno o más elementos de constructos genéticos conocidos per se, tal como por ejemplo, uno o más elementos reguladores adecuados (tal como promotores, mejoradores, terminadores adecuados, etc.)

y los elementos adicionales de constructos genéticos referidos en este documento anteriormente. Tales constructos genéticos que comprende al menos un ácido nucleico de la invención también serán referidos en este documento como "*constructos genéticos de la invención*".

Los constructos genéticos de la invención pueden ser ADN o ARN, y son preferiblemente ADN ramificados doble. Los constructos genéticos de la invención pueden también estar en la forma adecuada para transformación de la célula hospedante u organismo hospedante planeado, en una forma adecuada para integración en el ADN genómico de la célula hospedante planeada o en una forma de replicación independiente adecuada, mantenido y/o legado en el organismo hospedante planeado. Por ejemplo, los constructos genéticos de la invención pueden estar en la forma de un vector, tal como por ejemplo, un plásmido, cósmido, YAC, un vector viral o transposon. En particular, el vector puede ser un vector de expresión, es decir, un vector que puede ser proporcionado para expresión *in vitro* y/o *in vivo* (por ejemplo, en una célula hospedante, organismo hospedante y/o sistema de expresión adecuado).

En una modalidad preferida, pero no limitante, un constructo genético de la invención comprende

a) al menos un ácido nucleico de la invención; operablemente conectado a

b) uno o más elementos reguladores, tal como un promotor y opcionalmente un terminador adecuado;

c) y opcionalmente también

d) uno o más elementos adicionales de constructos genéticos conocidos per se;

en el cual los términos "elemento regulador", "promotor", "terminador" y "operablemente conectado" tiene su significado usual en la técnica (como se describe adicionalmente abajo); y en la cual dichos "elementos adicionales" presentes en los constructos genéticos pueden por ejemplo ser secuencias 3'- o 5'-URT, secuencias líder, marcadores de selección, marcadores de expresión/genes reporteros, y/o elementos que pueden facilitar o incrementar (la eficacia de) transformación o integración. Estos y otros elementos adecuados para tales constructos genéticos serán claros a una persona experta, y puede por ejemplo depender del tipo de constructo usado, la célula hospedante u organismo hospedante planeado; la manera en la cual las secuencias del nucleótido de interés, son para ser expresadas (por ejemplo, vía constitutiva, temporal o expresión inducible); y/o la técnica de transformación para ser usada.

Preferiblemente, en los constructos genéticos de la invención, al menos un ácido nucleótido de la invención y los elementos reguladores, y opcionalmente uno o más

elementos adicionales, son "operablemente enlazados" entre sí, por el cual es generalmente representado que están en una relación funcional entre sí. Por ejemplo, un promotor se considera "operablemente enlazado" a una secuencia codificante, si el promotor es capaz de iniciar o de otra forma controlar/regular las transcripción y/o la expresión de una secuencia codificante (en la cual la secuencia codificante debe entenderse por estar "bajo el control de" el promotor). Generalmente, cuando dos secuencias de nucleótido están operablemente enlazadas, pueden estar en la misma orientación y usualmente también en la misma estructura lectora. Usualmente también son esencialmente contiguos, aunque esto puede también no ser requerido.

Preferiblemente, los elementos reguladores y adicionales de los constructos genéticos de la invención son de tal forma que son capaces de proporcionar su función biológica planeada en la célula hospedante u organismo hospedante planeado.

Por ejemplo, un promotor, un intensificador o terminador debe ser "*operable*" en la célula hospedante u organismo hospedante, por el cual se piensa que (por ejemplo) el promotor debe ser capaz de iniciar o de otra forma controlar/regular la transcripción y/o la expresión de una secuencia de nucleótido, por ejemplo, una secuencia codificante, la cual está operablemente enlazada (como se

define en este documento).

Algunos promotores particularmente preferidos incluyen, pero no se limitan a, promotores conocidos per se para la expresión en células bacterianas, tales como aquellas mencionadas en este documento posteriormente y/o aquellas usadas en los Ejemplos.

Una selección de marcadores debe ser de tal forma que permita, por ejemplo, bajo condiciones de selección apropiadas, células hospedantes y/o organismos hospedantes que se han transformado (exitosamente) con la secuencia de nucleótido de la invención para ser distinguida de las células/organismos hospedantes que no se han transformado (exitosamente). Algunos ejemplos preferidos, pero no limitantes de tales marcadores, son genes que proporcionan resistencia contra antibióticos (tales como canamicina o ampicilina), genes que proporcionan resistencia a temperatura, o genes que permiten a la célula hospedante u organismo hospedante ser mantenidos en la ausencia de ciertos factores, compuestos y/o componentes (alimento) en el medio que son esencialmente para supervivencia de las células u organismos no transformados.

Una secuencia líder debe de ser de forma tal que, en la célula hospedante u organismo hospedante planeado, permite las modificaciones post-traduccional deseado y/o de forma tal que dirige el ARNm transcripto a una parte

deseada u organelo de una célula. Una secuencia líder puede también permitir la secreción del producto de expresión de la célula. Como tal, la secuencia líder puede ser pro-, pre- o prepo-secuencia operable en la célula hospedante u organismo hospedante. Las secuencias líderes no pueden ser requeridas para expresión de una célula bacteriana.

Un marcador de expresión o un gen reportero debe de ser de forma tal que en la célula hospedante u organismo hospedante, permita la detección de la expresión de (un gen o secuencia de nucleótido presente en) el constructo genético. Un marcador de expresión puede opcionalmente también permitir la localización del producto expresado, por ejemplo, en una parte específica u organelo de una célula y/o en células, tejidos, órganos o partes específicas de un organismo multicelular. Tales genes reporteros pueden también ser expresados como una fusión de proteína con la secuencia de aminoácido de la invención. Algunos ejemplos preferidos, pero no limitantes incluyen proteínas fluorescentes tales como GFP.

Algunos ejemplos preferidos, pero no limitantes de promotores, terminadores y elementos adicionales adecuados incluyen aquellos usados en los Ejemplos de abajo. Para algunos ejemplos no limitantes de los promotores, marcadores de selección, secuencias líderes, marcadores de expresión y elementos adicionales que pueden

estar presentes/usados en los constructos genéticos de la invención, tale como terminadores, intensificadores transcripcionales y/o traduccionales, y/o factores de integración, se hace referencia a los manuales generales tal como Sambrook et al., y Ausubel et al., mencionados anteriormente, así como para los ejemplos que se proporcionan en los documentos WO 95/07463, WO 96/23810, WO 95/07463, WO 95/21191, WO 97/11094, WO 97/42320, WO 98/06737, WO 98/21355, US-A-6,207,410, US-A-5,693,492 y EP 1 085 089. Otros ejemplos serán claros a la persona experta. Se hace referencia al antecedente general de la técnica citado anteriormente y las referencias citadas en este documento posteriormente.

Los constructos genéticos de la invención, pueden generalmente ser proporcionados para enlazar adecuadamente las secuencias del nucleótido de la invención a uno o más elementos adicionales descritos anteriormente, por ejemplo usando las técnicas descritas en los manuales generales tales como Sambrook et al., y Ausubel et al., mencionados anteriormente.

A menudo, los constructos genéticos de la invención serán obtenidos insertando una secuencia de nucleótido de la invención en un vector adecuado (expresión) conocido per se. Algunos ejemplos preferidos, no limitantes de vectores de expresión adecuados son

aquellos usados en los Ejemplos posteriores, así como aquellos mencionados debajo.

Los ácidos nucleicos de la invención y/o los constructos genéticos de la invención pueden ser usados para transformar una célula hospedante u organismo hospedante. El hospedante o célula hospedante, puede ser cualquier célula (fúngica, procariótica o eucariótica) o línea celular adecuada o cualquier organismo fúngico, procariótico o eucariótico adecuado, por ejemplo:

- una cepa bacteriana, que incluye pero no se limita a cepas gram negativo, tal como cepas de *Escherichia coli*; de *Proteus*, por ejemplo de *Proteus mirabilis*; de *Pseudomonas*, por ejemplo de *Pseudomonas fluorescens*; y cepas gram positivas tales como cepas de *Bacillus*, por ejemplo de *Bacillus subtilis* o de *Bacillus brevis*; de *Streptomyces*, por ejemplo de *Streptomyces lividans*; de *Staphylococcus*, por ejemplo de *Staphylococcus carnosus*; y de *Lactococcus*, por ejemplo de *Lactococcus lactis*;

- una célula fúngica, que incluye pero no se limita a células de especies de *Trichoderma*, por ejemplo de *Trichoderma reesei*; de *Neurospora*, por ejemplo de *Neurospora crassa*; de *Sordaria*, por ejemplo de *Sordaria macrospora*; de *Aspergillus*, por ejemplo de *Aspergillus niger* o de *Aspergillus sojae*; o de otros hongos filamentosos;

- una célula de levadura, que incluye pero no se limita a células de especies de *Saccharomyces*, por ejemplo de *Saccharomyces cerevisiae*; de *Schizosaccharomyces*, por ejemplo de *Schizosaccharomyces pombe*; de *Pichia*, por ejemplo de *Pichia pastoris* o de *Pichia methanolica*; de *Hansenula*, por ejemplo de *Hansenula polymorpha*; de *Kluyveromyces*, por ejemplo de *Kluyveromyces lactis*; de *Arxula*, por ejemplo de *Arxula adeninivorans*; de *Yarrowia*, por ejemplo de *Yarraowia lipolytica*;

- una célula anfibiana o línea celular, tal como *Xenopus oocytes*;

- una célula o línea celular derivada de insecto, tal como células/líneas celulares derivadas de lepidóptera, que incluyen pero no se limita a células SF9 y Sf21 de *Spodoptera* o células/líneas celulares derivadas de *Drosophila*, tal como células de Schneider y Kc;

- una planta o célula de planta, por ejemplo en plantas de tabaco; y/o

- una célula o línea celular de mamífero, por ejemplo derivado de una célula o línea celular derivada de un humano; a partir de mamíferos que incluyen pero no se limitan a células CHO, células BHK (por ejemplo células BHK-21) y células o líneas celulares humanas como HeLa, COS (por ejemplo COS-7) y células PER.C6;

así como otros hospedantes o células hospedantes conocidas

per se, para la expresión y producción de anticuerpos y fragmentos de anticuerpo (que incluyen, pero no se limita a anticuerpos de dominio (único) y fragmentos ScFv), los cuales serán claros a la persona experta. También se hace referencia al antecedente general de la técnica citado en este documento anteriormente, así como por ejemplo en el documento WO 94/29457; documento WO 96/34103; WO 99/42077; Frenken et al., (1998), supra; Riechmann y Muyldermans, (1999), suptra; van der Linden, (2000), suptra; Thomassen et al., (2002), suptra; Joosten et al., (2003), supra; Joosten et al., (2005), suptra; y las referencias adicionales citadas en este documento.

Los Nanocuerpos y polipéptidos de la invención, pueden también ser introducidos y expresados en una o más células, tejidos u órganos de un organismo multicelular, por ejemplo, para propósitos profilácticos y/o terapéuticos (por ejemplo, como una terapia de gen). Para este propósito, las secuencias de nucléotido de la invención pueden ser introducidas en las células o tejidos, en cualquier forma adecuada, por ejemplo tal como (por ejemplo, usando liposomas) o después que se han insertado en un vector de terapia de gen adecuado (por ejemplo, derivado de retrovirus tal como adenovirus, o parvovirus tal como virus adeno-asociado). También será claro a la persona experta, tal terapia de gen puede ser realizada *in*

vivo y/o *in situ* en el cuerpo de un paciente, administrando un ácido nucleico de la invención o un vector de terapia de gen adecuada, que codifica lo mismo al paciente o a células específicas o, un tejido u órgano específico del paciente; o células adecuadas (a menudo tomadas del cuerpo del paciente a ser tratado, tal como linfocitos colocados en medio de cultivo, aspirados de médula ósea o biopsias de tejido) pueden ser tratados en el cuerpo del paciente. Todos esto puede ser realizado usando vectores, técnicas y sistemas de suministro de terapia de gen, los cuales son bien conocidos por la persona experta, por Culver, K. W., "Gene Therapy", 1994, p. xii, Mary Ann Liebert, Inc., Publishers, New York, N.Y.). Giordano, Nature F Medicine 2 (1996), 534-539; Schaper, Circ. Res. 79 (1996), 911-919; Anderson, Science 256 (1992), 808-813; Verma, Nature 389 (1994), 239; Isner, Lancet 348 (1996), 370-374; Muhlhauser, Circ. Res. 77 (1995), 1077-1086; Onodera, Blood 91; (1998), 30-36; Verma, Gene Ther. 5 (1998), 692-699; Nabel, Ann. N.Y. Acad. Sci.: 811 (1997), 289-292; Verzeletti, Hum. Gene Ther. 9 (1998), 2243-51; Wang, Nature Medicine 2 (1996), 714-716; WO 94/29469; WO 97/00957, US 5,580,859, 1 US 5,5895466; o Schaper, Current Opinion in Biotechnology 7 (1996), 635-640. Por ejemplo, expresión in situ de fragmentos ScFv (Afanasieva et al., Gene Ther., 10, 1850-1859 (2003)) y de diacuerpos (Blanco et al., J. Immunol,

171, 1070-1077 (2003)) se han descrito en la técnica.

Para expresión de los Nanocuerpos en una célula, pueden ser también expresados como son llamados "intracuerpos", como por ejemplo se describen en los documentos WO 94/02610, WO 95/22618 y US-A-7004940; WO 03/014960; en Cattaneo, A. & Biocca, S. (1997) Intracellular Antibodies: Development and Applications. Landes and Springer-Verlag; y en Kontermann, Methods 34, (2004), 163-170.

Para producción, los Nanocuerpos y polipéptidos de la invención, pueden por ejemplo también ser producidos en la leche de mamíferos transgénicos, por ejemplo, en la leche de conejos, vacas, gatos u ovejas (véase por ejemplo, los documentos US-A-6,741,957, US-A-6,304,489 y US-A-6,849,992 para generar técnicas para introducir transgenes en mamíferos), en plantas o partes de plantas, que incluyen pero no se limita a sus hojas, flores, frutos, semillas, raíces o tubérculos (por ejemplo, en tabaco, maíz, fríjol de soya o alfalfa) o en por ejemplo, pupa del gusano de seda *Bombix mori*.

Además, los Nanocuerpos y polipéptidos de la invención, pueden también ser expresados y/o ser producidos en sistemas de expresión de célula libre, y ejemplos adecuados de tales sistemas serán claros a la persona experta. Algunos ejemplos preferidos, pero no limitantes

incluyen expresión en el sistema de germen de trigo; en lisados reticulocito de conejo; o en el sistema Zubay de *E. coli*.

Como se menciona anteriormente, una de las ventajas del uno de Nanocuerpos es que los polipéptidos en base al respecto, pueden ser preparados a través de la expresión en un sistema bacteriano adecuado, y sistemas de expresión bacteriana adecuada, vectores, células hospedantes, elementos reguladores, etc., será claro a la persona experta, por ejemplo a partir de las referencias citadas anteriormente. Se notará sin embargo que la invención en su sentido más amplio no se limita a expresión en sistemas bacteriana.

Preferiblemente, en la invención, un sistema de expresión (in vivo o in vitro), tal como un sistema de expresión bacteriano, se usa para proporcionar los polipéptidos de la invención en una forma que es adecuada para uso farmacéutico, y tales sistemas de expresión nuevamente serán claros a la persona experta. También será claro a la persona experta, se pueden preparar polipéptidos de la invención adecuados para uso farmacéutico usando técnicas para síntesis de péptido.

Para producción en escala industrial, hospedantes heterólogos preferidos para la producción (industrial) de Nanocuerpos o Nanocuerpo que contiene terapéuticos de

proteína incluyen cepas de *E. coli*, *Pichia pastoris*, *S. cerevisiae*, que son adecuados para expresión/producción/fermentación a gran escala, y en particular para expresión/producción/fermentación farmacéutica a gran escala. Ejemplos adecuados de tales cepas serán claros a la persona experta. Tales cepas y sistemas de producción/expresión también están disponibles por compañías tal como Biovitrum (Uppsala, Suecia).

Alternativamente, líneas celulares de mamífero, en particular células de ovario (CHO) de hámster Chino pueden ser usadas para expresión/producción/fermentación a gran escala, y en particular para expresión/producción/fermentación farmacéutica a gran escala. Nuevamente, tales sistemas de expresión/producción también están disponibles por algunas de las compañías mencionadas anteriormente.

La selección del sistema de expresión específico dependerá en parte en el requerimiento para ciertas modificaciones post-traduccional, más específicamente glicosilación. La producción de un Nanocuerpo que contiene proteína recombinante para la cual la glicosilación deseada o requerida necesitará el uso de hospedante de expresión en mamífero, que tiene la capacidad para glicosilar la proteína expresada. En este respecto, será claro a la persona experta que el patrón de glicosilación obtenido (es

decir, la clase, número y posición de residuos unidos) dependerá en la célula o línea celular que se usa para la expresión. Preferiblemente, ya sea una célula humana o línea celular usada (es decir, que conduce a una proteína que esencialmente tiene un patrón de glicosilación humano) o se usa otra línea celular de mamífero que puede proporcionar un patrón de glicosilación que es esencialmente y/o funcionalmente el mismo como glicosilación humana o al menos glicosilación humana imitadora. Generalmente, los hospedantes procarióticos tal como *E. coli* no tienen la capacidad para glicosilar proteínas, y el uso de eucariotas inferiores tal como levadura, son usualmente conducidos a patrón de glicosilación que difiere de glicosilación humana. Sin embargo, se entenderá que todas las células hospedantes extrañas y sistemas de expresión se pueden usar en la invención, dependiendo del Nanocuerpo o proteína deseada a ser obtenida.

Así, de conformidad a una modalidad no limitante de la invención, el Nanocuerpo o polipéptido de la invención es glicosilado. De acuerdo a otra modalidad no limitante de la invención, el Nanocuerpo o polipéptido de la invención no es glicosilado.

De acuerdo a una modalidad preferida, no limitante de la invención, el Nanocuerpo o polipéptido de

la invención se produce en una célula bacteriana, en particular una célula bacteriana adecuada para producción farmacéutica a gran escala, tales células de las cepas mencionadas anteriormente.

De acuerdo a otra modalidad preferida, no limitante de la invención, el Nanocuerpo o polipéptido de la invención se produce en una célula de levadura, en particular una célula de levadura adecuada para producción farmacéutica a gran escala, tal como células de las especies mencionadas anteriormente.

De acuerdo a aún otra modalidad preferida, pero no limitante de la invención, el Nanocuerpo o polipéptido de la invención se produce en una célula de mamífero, en particular en una célula humana o en una línea celular humana, y más en particular en una célula humana o en una célula de una línea celular humana que es adecuada para producción farmacéutica a gran escala, tal como las líneas celulares mencionadas anteriormente.

Cuando la expresión en una célula hospedante se usa para producir los Nanocuerpos y las proteínas de la invención, los Nanocuerpos y proteínas de la invención pueden ser producidos ya sea intracelularmente (por ejemplo, en el cistol, en el plasma o en cuerpos de inclusión) y después aislado a partir de las células hospedantes y opcionalmente purificados adicionalmente; o

puede ser producido extracelularmente (por ejemplo, en el medio en el cual las células hospedantes se cultivan) y después aislado a partir del medio de cultivo y opcionalmente purificado adicionalmente. Cuando se usan células hospedantes eucarióticas, la producción extracelular es usualmente preferida ya que esta considerablemente facilita el aislamiento adicional y proceso de corriente abajo de los Nanocuerpos y proteínas obtenidas. Células bacterianas tales como las cepas de *E. coli* mencionada anteriormente normalmente no secretan proteínas extracelular, excepto por unas pocas clases de proteínas tal como toxinas y hemolisina, y producción secretoria en *E. coli* se refiere a la translocación de proteínas a través de la membrana interior para el espacio periplásmico. La producción de periplásmico proporciona varias ventajas sobre la producción citosólica. Por ejemplo, la secuencia de aminoácido N-terminal del producto secretado, puede ser idéntico al producto del gen natural después de desdoblar la secuencia de señal de secreción por una peptidasa de señal específico. También, parece tener mucha menos actividad de proteasa en el periplasma que en el citoplasma. Además, la purificación de proteína es simple debido a pocas proteínas que contaminan en el periplasma. Otra ventaja es que uniones de disulfuro correctas pueden ser formadas debido al periplasma,

proporciona un ambiente más oxidativo que el citoplasma. Las proteínas expresadas en *E. coli* son a menudo expresadas en agregados insolubles, así llamados cuerpos de inclusión. Estos cuerpos de inclusión pueden ser localizados en el cistol o en el periplasma; la recuperación de proteínas biológicamente activas a partir de estos cuerpos de inclusión requiere un proceso de desnaturalización/repliegue. Muchas proteínas recombinantes, que incluyen proteínas terapéuticas, se recuperan a partir de cuerpos de inclusión. Alternativamente, será claro a una persona experta, que las cepas recombinantes de bacteria que se han modificado genéticamente para secretar una proteína deseada, y en particular se puede usar un Nanocuerpo o un polipéptido de la invención.

Así, de acuerdo a una modalidad no limitante de la invención, el Nanocuerpo o polipéptido de la invención es un Nanocuerpo o polipéptido que se han producido intracelularmente y que se han aislado de la célula hospedante, y en particular de una célula bacteriana o de un cuerpo de inclusión en una célula bacteriana. De acuerdo a otra modalidad no limitante de la invención, el nanocuerpo o polipéptido de la invención es un Nanocuerpo o polipéptido que se ha producido extracelularmente, y que se ha aislado del medio el cual se cultiva la célula

hospedante.

Algunos promotores preferidos, no limitantes para uso con estas células hospedantes incluyen,

- para expresión en *E. coli*: promotor lac (y derivados de los mismos, tal como el promotor lacUV5); promotor arabinosa; promotor hacia la izquierda- (PL) y hacia la derecha (PR) del fago lambda; promotor del operon trp; promotores lac/trp híbridos (tac y trc); promotor T7 (más específicamente del gen 10 de fago T7) y otros promotores de fago T; promotor del gen resistente de tetraciclina Tn10; variantes diseñados por ingeniería de los promotores anteriores que incluyen una o más copias de una secuencia operadora reguladora superflua;

- para expresión en S. cerevisiae: constitutivo: ADH1 (deshidrogenasa de alcohol 1), ENO (enolasa), CYC1 (citocromo c iso-1), GAPDH (deshidrogenasa gliceraldehído-3-fosfato); PGK1 (fosfoglicerato cinasa), PYK1 (piruvato cinasa); regulado: GAL1,10,7 (enzimas metabólicas de galactosa), ADH2 (deshidrogenasa de alcohol 2), PHO5 (fosfatasa de ácido), CUP1 (metalotioneina de cobre); heterólogos: CaMV (promotor 35S del virus de mosaico de coliflor);

- para expresión en *Pichia pastoris*: el promotor AOX1 (oxidasa de alcohol I)

- para expresión en células de mamífero:

intensificador/promotor inmediato temprano de citomegalovirus humano (hCMV); variante del promotor inmediato temprano del citomegalovirus humano (hCMV) que contiene dos secuencias operadoras de tetraciclina, de forma tal que el promotor puede ser regulado por el represor Tet; promotor de timidina cinasa (TK) del Virus de Herpes Simple; intensificador/promotor de terminal repetida largo (RSV LTR) del Virus de Sarcoma Rous; promotor del factor 1α de alargamiento (heF-1α) a partir de humano, chimpancé, ratón o rata; el promotor temprano SV40; promotor de terminal repetida larga HIV-1; promotor β-actina;

Algunos vectores preferidos, pero no limitantes para uso con estas células hospedantes incluyen:

- vectores para expresión en células de mamífero: pMAMneo (Clontech), pcADN3 (Invitrogen), pMC1neo (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593), pBPV-1 (8-2) (ATCC 37110), pdBPV-MMTneo (342-12) (ATCC 37224), pRSVgpt (ATCC37199), pRSVneo (ATCC37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460) y 1ZD35 (ATCC 37565), así como sistemas de expresión en base a virus, tal como aquellos en base a adenovirus;

- vectores para expresión en células bacterianas: vectores pET (Novagen) y vectores pQE (Qiagen);

- vectores para expresión en levadura u otras

células fúngicas: pYES2 (Invitrogen) y vectores de expresión en *Pichia* (Invitrogen);

- vectores para expresión en células de insecto: pBlueBacII (Invitrogen) y otros vectores de baculovirus;

- vectores para expresión en plantas o células de planta: por ejemplo vectores en base a virus del mosaico de coliflor o virus del mosaico de tabaco, cepas adecuadas de *Agrobacterium*, o vectores en base a plásmido Ti.

También secuencias secretorias preferidos, pero no limitantes para uso con estas células hospedantes incluyen:

- para uso en células bacterianas tales como *E. coli*: PelB, Bla, OmpA, OmpC, OmpF, OmpT, StII, PhoA, PhoE, MalE, Lpp, LamB y similares; péptido de señal TAT, señal secretoria C-terminal de hemolisina;

- para uso en levadura: prepo-secuencia de factor α-apareamiento, fosfatasa (pho1), invertasa (Suc), etc.;

- para uso en células de mamífero: señal indígena en caso de la proteína objetivo es de origen eucariótico; péptido de señal V-J2-C cadena κ murina Ig; etc.

Técnicas adecuadas para transformar un hospedante o célula hospedante de la invención será claro a una persona experta y puede depender en el organismo célula hospedante/hospedante planeado y el constructo genético a ser usado. Nuevamente se hace referencia a los manuales y

solicitudes de patente mencionados anteriormente.

Después de la transformación, se puede realizar una etapa para detectar y seleccionar aquellas células hospedantes u organismos hospedantes que han sido exitosamente transformados con la secuencia de nucleótido/constructo genético de la invención. Esto puede por ejemplo ser una etapa de selección en base a marcadores seleccionables presentes en el constructo genético de la invención o una etapa que involucra la detección de la secuencia de aminoácido de la invención, por ejemplo, usando anticuerpos específicos.

La célula hospedante transformada (la cual puede estar en la forma o una línea celular estable) u organismos hospedantes (los cuales puede estar en la forma de una línea o cepa mutante adecuada) forman aspectos adicionales de la presente invención.

Preferiblemente, estas células hospedantes u organismos hospedantes son de tal forma que expresan, o son capaces (al menos) de expresar (por ejemplo, bajo condiciones adecuados), una secuencia de aminoácido de la invención (y en caso de un organismo hospedante: en al menos una célula, tejido u órgano del mismo). La invención también incluye generaciones, progenie y/o descendencia adicional de la célula hospedante u organismo hospedante de la invención, que puede por ejemplo ser obtenido por

división celular o por reproducción sexual o asexual.

Para producir/obtener expresión de las secuencias de aminoácido de la invención, la célula hospedante transformada u organismo hospedante transformado puede generalmente ser conservado, mantenido y/o cultivado bajo condiciones de forma tal que la secuencia de aminoácido (deseada) de la invención es expresada/producida. Serán claras condiciones adecuadas a la persona experta y usualmente dependerá de la célula hospedante/organismo hospedante usado, así como en los elementos reguladores del control de expresión de la secuencia de nucleótido (relevante) de la invención. Nuevamente, se hace referencia a los manuales y solicitudes de patente mencionadas anteriormente en los párrafos en los constructos genéticos de la invención.

Generalmente, condiciones adecuadas pueden incluir el uso de un medio adecuado, la presencia de una fuente adecuada de alimento y/o nutrientes adecuada, el uso de una temperatura adecuada, y opcionalmente la presencia de un factor o compuesto de inducción adecuado (por ejemplo, cuando las secuencias del nucléotido de la invención están bajo el control de un promotor inducible); todos los cuales pueden ser seleccionados por la persona experta. Nuevamente, bajo tales condiciones, las secuencias de aminoácido de la invención pueden ser expresadas en una

manera constitutiva, en una manera temporal, o únicamente cuando es adecuadamente inducido.

También será claro a una persona experta que la secuencia de aminoácido de la invención, puede (primero) ser generado en una forma inmadura (como se menciona anteriormente), la cual después se somete a modificación post-traduccional, dependiendo de la célula hospedante/organismo hospedante usado. También, la secuencia de aminoácido de la invención puede ser glicosilado, nuevamente dependiendo de la célula hospedante/organismo hospedante usado.

La secuencia de aminoácido de la invención, puede después ser aislada de la célula hospedante/organismo hospedante y/o del medio en cual la célula hospedante u organismo hospedante se cultiva, usando técnicas de aislamiento y/o purificación de proteína conocidas per se, tal como técnicas de cromatografía (preparativa) y/o electroforesis, técnicas de precipitación diferencial, técnicas de afinidad (por ejemplo, usando una secuencia de aminoácido desdoblado, específico fusionado con la secuencia de aminoácido de la invención) y/o técnicas inmunológicas preparativas (es decir, usando anticuerpos contra la secuencia de aminoácido a ser aislado).

Generalmente, para uso farmacéutico, los polipéptidos de la invención pueden ser formulados como una

preparación farmacéutica que comprende al menos un polipéptido de la invención y al menos un portador diluyente o excipiente y/o adyuvante farmacéuticamente aceptable, y opcionalmente uno o más polipéptidos y/o compuestos farmacéuticamente activos adicionales. Por medio de ejemplos no limitantes, tal como una formulación puede estar en una forma adecuada para administración oral, para administración parenteral (tal como por inyección intravenosa, intramuscular o subcutánea o infusión intravenosa), para administración tópica, para administración por inhalación, por un parche de piel, por un implante, por un supositorio, etc. Tales formas de administración adecuadas, las cuales pueden ser sólidas, semi-sólidas o líquidas, dependiendo de la forma de administración, así como métodos y portadores para uso en la preparación del mismo será claro a una persona experta, y se describen adicionalmente en este documento posteriormente.

Generalmente, los Nanocuerpo y polipéptidos de la invención pueden ser formulados y administrados en una manera adecuada conocida per se, para lo cual se hace referencia por ejemplo al antecedente general de la técnica citado anteriormente (y en particular a los documentos WO 04/041862, WO 04/041863, WO 04/041865 y WO 04/041867) así como a los manuales estándar, tal como Remington's

99B

Pharmaceutical Sciences, 18ava Edición, Mack Publishing Company, USA (1990) o Remington, the Science and Practice of Pharmacy, 21ava Edición, Lippincott William and Wilkins (2005).

Por ejemplo, los Nanocuerpos y polipéptidos de la invención pueden ser formulados y administrados en una manera conocida per se, para anticuerpos convencionales y fragmentos de anticuerpo (que incluyen ScFv's y diacuerpos) y otras proteínas farmacéuticamente activas. Tales formulaciones y métodos para preparar la misma, será claro a una persona experta, y por ejemplo incluye preparaciones adecuadas para administración parenteral (por ejemplo, administración intravenosa, intraperitoneal, subcutánea, intramuscular, intraluminal, intra-arterial o intratecal) o para administración tópica (es decir, transdérmica o intradérmica).

Las preparaciones para administración parenteral pueden por ejemplo ser soluciones estériles, suspensiones, dispersiones o emulsiones que son adecuadas para infusión o inyección. Portadores o diluyentes adecuados para tales preparaciones por ejemplo incluyen, sin limitación, agua estéril y amortiguadores acuosos y soluciones tales como salina amortiguada de fosfato fisiológica, soluciones de Ringer, solución dextrosa y solución de Hank; aceites acuoso; etanol; glicoles tales como propilen glicol o así

como aceites minerales, aceites de animal y aceites vegetales, por ejemplo, aceite de cacahuate, aceite de fríjol de soya, así como mezclas adecuadas de los mismos. Usualmente, se pueden preferir soluciones o suspensiones acuosas.

Los Nanocuerpos y polipéptidos de la invención, también pueden ser administrados usando métodos de suministro de terapia de gen. Véase, por ejemplo, la Patente Estadounidense No. 5,399,346, la cual se incorpora por referencia en su totalidad. Usando un método de suministro de terapia de gen, células primarias transfectadas con el gen que codifica un Nanocuerpo o polipéptido de la invención pueden adicionalmente ser transfectadas con promotores específicos del tejido para órganos específicos objetivo, tejido, injertos, tumores o células, y pueden adicionalmente ser transfectadas con secuencias señal y de estabilización para expresión subcelularmente localizadas.

Así, los nanocuerpos y polipéptidos de la invención pueden ser sistemáticamente administrados, por ejemplo, oralmente, en combinación con un vehículo farmacéuticamente aceptable tal como un diluyente inerte o un portador comestible asimilable. Pueden estar juntos en cápsulas de gelatina de cubierta suave o dura, pueden ser comprimidos en tabletas, o pueden ser incorporados

directamente con el alimento en la dieta del paciente. Para administración terapéutica oral, los Nanocuerpos y polipéptidos de la invención pueden ser combinados con uno o más excipientes y usados en la forma de tabletas ingeribles, tabletas bucales, trociscos, cápsulas, elixires, suspensiones, jarabes, obleas y similares. Tales composiciones y preparaciones deben contener al menos 0.1% del Nanocuerpo o polipéptido de la invención. El porcentaje de las composiciones y preparaciones pueden, por supuesto, ser variados y pueden convenientemente ser entre aproximadamente 2 hasta aproximadamente 60% del peso de una forma de dosificación unitaria proporcionada. La cantidad del Nanocuerpo o polipéptido de la invención en tales composiciones terapéuticamente útiles es de forma tal que se obtiene un nivel de dosificación efectivo.

Las tableas, trociscos, píldoras, cápsulas y similares, pueden también contener lo siguiente: aglutinantes tales como goma de tragacanto, acacia, almidón de maíz o gelatina; excipientes tales como fosfato dicalcio; un agente desintegrante tal como almidón de maíz, almidón de papa, ácido algínico y similares; un lubricante tal como estearato de magnesio; y un agente edulcorante tal como sacarosa, fructuosa, lactosa o aspartame o se puede agregar un agente saborizante tal como menta, aceite de gaulteria, o saborizante a cereza. Cuando una forma de

dosificación unitaria es una cápsula, puede contener, además a materiales del tipo anterior, un líquido portador, tal como un aceite vegetal o un polietilen glicol. Otros materiales variados pueden estar presentes como revestimientos o para de otra forma modificar la forma física de la forma de dosificación unitaria sólida. Por ejemplo, tabletas, píldoras o cápsulas pueden ser revestidos con gelatina, cera, laca o azúcar y similares. Un jarabe o elixir puede contener los Nanocuerpos y polipéptidos de la invención, sacarosa o fructuosa como un agente edulcorante, metilo y propilparabenos como preservativos, un tinte y saborizantes tales como sabor a cereza o naranja. Por supuesto, cualquier material usado en la preparación de cualquier dosificación unitaria debe ser farmacéuticamente aceptable y sustancialmente no tóxico en las cantidades empleadas. Además, los Nanocuerpos y polipéptidos de la invención pueden ser incorporados en preparaciones y dispositivos de liberación sostenida.

También se pueden proporcionar preparaciones y formulaciones para administración oral con un revestimiento entérico que permitirá a los constructos de la invención resistir el ambiente gástrico y pasa a los intestinos. Más generalmente, las preparaciones y formulaciones para administración oral pueden ser adecuadamente formuladas para suministro en cualquier parte deseada del tracto

997

gastrointestinal. Además, se puede usar supositorios adecuados para suministro en el tracto gastrointestinal.

Los Nanocuerpos y polipéptidos de la invención pueden también ser administrados intravenosamente o intraperitonealmente por infusión o inyección. Las soluciones de los Nanocuerpos y polipéptidos de la invención o sus sales se pueden preparar en agua, opcionalmente mezclado con un tensoactivo no tóxico. También se pueden preparar dispersiones en glicerol, polietilen glicoles líquidos, triacetina y mezclas de los mismos y en aceites. Bajo condiciones ordinarias de almacena y uso, estas preparaciones contienen un preservativo para prevenir el crecimiento de microorganismos.

Las formas de dosificación farmacéuticas adecuadas para inyección o infusión pueden incluir soluciones o dispersiones acuosas estériles o polvos estériles que comprenden el ingrediente activo, los cuales están adaptados para la preparación extemporánea de inyectables estériles o, soluciones o dispersiones infundibles, opcionalmente encapsulados en liposomas. En todos los casos, la última forma de dosificación puede ser estéril, fluida y estable bajo las condiciones de manufactura y almacenaje. El portador o vehículo líquido puede ser un solvente o un medio de dispersión líquido que

comprende, por ejemplo, agua, etanol un poliol (por ejemplo, glicerol, propileno, glicol, polietilen glicoles líquidos y similares), aceites vegetales, gliceril éteres no tóxicos y mezclas de los mismos adecuadas. La fluidez apropiada puede ser mantenida, por ejemplo, por la formación de liposomas, por el mantenimiento del tamaño de partícula requerido en el caso de dispersiones o por el uso de tensoactivos. La prevención de acción de microorganismos puede ser iniciar aproximadamente por varios agentes antibacterianos y antifúngicos, por ejemplo, parabenos, clorobutanol, fenol, ácido sórbico, timerosal y similares. En muchos casos, será preferible incluir agentes isotónicos, por ejemplo, azúcares, amortiguadores o cloruro de sodio. Se puede iniciar la absorción prolongada de las composiciones inyectables aproximadamente por el uso en las composiciones de agentes que retrasan la absorción, por ejemplo, monoestearato de aluminio y gelatina.

Se preparan soluciones inyectables estériles incorporando los Nanocuerpos y polipéptidos de la invención en la cantidad requerida en el solvente apropiado con varios de los otros ingredientes enumerados anteriormente, como se requiere, seguido por esterilización por filtración. En el caso de polvos estériles para la preparación de soluciones inyectables estériles, los métodos preferidos de preparación son secado a vacío y

técnicas de liofilizado, las cuales proporcionan un polvo del ingrediente activo más cualquier ingrediente deseado adicional presente en las soluciones filtradas, previamente esterilizadas.

Para administración tópica, los Nanocuerpos y polipéptidos de la invención pueden ser aplicadas en forma pura, es decir, cuando son líquidos. Sin embargo, será generalmente deseable administrarlos a la piel como composiciones o formulaciones, en combinación con un portador dermatológicamente aceptable, el cual puede ser un sólido o un líquido.

Portadores sólidos útiles incluyen, sólidos finalmente divididos tal como talco, arcilla, celulosa microcristalina, sílice, alúmina y similares. Portadores líquidos útiles incluyen agua, hidroxialquilo o glicoles, o mezclas de agua-alcohol/glicol, en las cuales los Nanocuerpos y polipéptidos de la invención pueden ser disueltos o dispersados en niveles efectivos, opcionalmente con la ayuda de tensoactivos no tóxicos. Se pueden agregar adyuvantes tal como fragancias y agentes antimicrobianos adicionales para optimizar las propiedades para un uso proporcionado. Las composiciones líquidas resultantes pueden ser aplicadas de almohadillas absorbentes, usadas para impregnar vendajes y otras preparaciones de vendaje, o rociadas en el área afectada usando rociadores en aerosol o

1000

tipo bomba.

También se pueden emplear espesantes tales como polímeros, ácidos grasos, sales de ácidos grasos y ésteres, alcoholes grasos, celulosas modificadas o materiales minerales modificados con portadores líquidos para formar pastas que se puede esparcir, geles, ungüentos, jabones y similares, para aplicación directamente a la piel del usuario.

Se conocen en la técnica ejemplos de composiciones dermatológicas útiles, las cuales se pueden usar para suministrar los Nanocuerpos y polipéptidos de la invención a la piel; por ejemplo, véase Jacquet et al., (Patente Estadounidense No. 4,608,392), Geria (Patente Estadounidense No. 4,992,478), Smith et al. (Patente Estadounidense No. 4,559,157) y Wortzman (Patente Estadounidense No. 4,820,508).

Dosificaciones útiles de los Nanocuerpos y polipéptidos de la invención se pueden determinar comparando su actividad *in vitro*, y actividad *in vivo* en modelos de animal. Se conocen métodos para la explotación de dosificaciones efectivas en ratones y otros animales, a humanos; por ejemplo, véase Patente Estadounidense No. 4,938,949.

Generalmente, la concentración de los Nanocuerpos y polipéptidos de la invención en una composición líquida,

1001

tal como una loción, será en la forma de aproximadamente 0.1-25 % en peso, preferiblemente aproximadamente 0.5-10% en peso. La concentración en una composición sólida o semi-sólida tal como un gel o un polvo será aproximadamente 0.1-5% en peso, preferiblemente 0.5-2.5% en peso.

La cantidad de los Nanocuerpos y polipéptidos de la invención requerida para uso en tratamiento, variará no únicamente con el Nanocuerpo o polipéptido particular seleccionado, pero también con la ruta de administración, la naturaleza de la condición a ser tratada y, la edad y condición del paciente y será en última instancia en la discreción del especialista o médico tratante. También la dosificación de los Nanocuerpos y polipéptidos de la invención varia dependiendo de la célula objetivo, tumor, tejido, injerto u órgano.

La dosis deseada puede convenientemente ser presentada en una dosis única o como dosis dividida administrada a intervalos apropiados, por ejemplo, como dos, tres, cuatro o más sub-dosis por día. La misma sub-dosis puede ser además dividida, por ejemplo, en un número de administraciones espaciadas con holgura discreta; tal como inhalaciones múltiples de un insuflador o por aplicación de una pluralidad de gotas al ojo.

Un régimen de administración puede incluir tratamiento diario, prolongado. Por "prolongado" significa

al menos dos semanas y preferiblemente, varias semanas, meses o años de duración. Se pueden determinar modificaciones necesarias en este intervalo de dosificación por un experto ordinario en la técnica, usando únicamente experimentación de rutina que proporciona las enseñanzas en este documento. Véase Remington's Pharmaceutical Sciences (Martin, E.W., ed. 4), Mack Publishing Co., Easton, PA. La dosificación también se puede ajustar por el especialista individual en el evento de cualquier complicación.

En otro aspecto, la invención se refiere a un método para la prevención y/o tratamiento de al menos una enfermedad o trastorno relacionado a TNF como se menciona en este documento, el método comprende administrar, a un sujeto en necesidad del mismo, una cantidad farmacéuticamente activa de un Nanocuerpo de la invención, de un polipéptido de la invención y/o de una composición farmacéutica que comprende el mismo.

En el contexto de la presente invención, el término "prevención y/o tratamiento" no únicamente comprende prevenir y/o tratar la enfermedad, pero también generalmente comprende prevenir el ataque de la enfermedad, disminuir o invertir el progreso de la enfermedad, prevenir o disminuir el ataque de uno o más síntomas asociados con la enfermedad, reducir y/o aliviar uno o más síntomas asociados con la enfermedad, reducir la severidad y/o la

duración de la enfermedad y/o de cualquiera de los síntomas asociados con esta y/o prevenir un incremento adicional en la severidad de la enfermedad y/o de cualquier síntoma asociado con esta, prevenir, reducir o invertir cualquier lesión fisiológica causada por la enfermedad, y generalmente cualquier acción que es benéfica para el paciente a ser tratado.

El sujeto a ser tratado puede ser cualquier animal de sangre caliente, pero es en particular un mamífero, y más en particular es un humano. Como será claro a la persona experta, el sujeto a ser tratado será en particular una persona que sufre de, o está en riesgo de, las enfermedades o trastornos mencionados en este documento.

La invención también se refiere a un método para la prevención y/o tratamiento de al menos una enfermedad o trastorno que puede ser prevenida y/o tratada administrando un Nanocuerpo o polipéptido de la invención a un paciente, el método comprende administrar a un sujeto en necesidad del mismo, una cantidad farmacéuticamente activa de un Nanocuerpo de la invención, de un polipéptido de la invención, y/o una composición farmacéutica que comprende el mismo.

Más en particular, la invención se refiere a un método para la prevención y/o tratamiento de al menos una

1004

enfermedad o trastorno escogido de un grupo que consiste de las enfermedades o trastornos listados en este documento, el método comprende administrar, a un sujeto en necesidad del mismo, una cantidad farmacéuticamente activa de un Nanocuerpo de la invención, de un polipéptido de la invención y/o de una composición farmacéutica que comprende el mismo.

En otra modalidad, la invención se refiere a un método para inmunoterapia, y en particular para inmunoterapia pasiva, dicho método comprende administrar, a un sujeto que sufre de o está en riesgo de las enfermedades y trastornos mencionados en este documento, una cantidad farmacéuticamente activa de un Nanocuerpo de la invención, de un polipéptido y/o de una composición farmacéutica que comprende el mismo.

En los métodos anteriores, los Nanocuerpos y/o polipéptidos de la invención y/o las composiciones que comprende el mismo, puede ser administrada en cualquier manera adecuada, dependiendo de la formulación o composición farmacéutica específica a ser usada. Así, los Nanocuerpos y/o polipéptidos de la invención y/o las composiciones que comprenden el mismo, pueden por ejemplo ser administradas oralmente, intraperitonealmente (por ejemplo, intravenosamente, subcutáneamente, intramuscularmente, o vía cualquier otra ruta de

1005

administración que evita el tracto gastrointestinal), intranasalmente, transdérmicamente, tópicamente, por medio de un supositorio, por inhalación, nuevamente dependiendo de la formulación o composición farmacéutica específica a ser usada. El médico será capaz de seleccionar una ruta adecuada de administración y una formulación o composición farmacéutica adecuada a ser usada en tal administración, dependiendo de la enfermedad o trastorno a ser prevenido o tratado y otros factores bien conocidos por el médico.

Los Nanocuerpos y/o polipéptidos de la invención y/o las composiciones que comprenden los mismos, son administradas de conformidad a un régimen de tratamiento que es adecuado para prevenir y/o tratar la enfermedad o trastorno a ser prevenido o tratado. El médico generalmente será capaz para determinar un régimen de tratamiento adecuado, dependiendo de factores tales como la enfermedad o trastorno a ser prevenidos o tratados, la severidad de la enfermedad a ser tratada y/o la severidad de los mismos síntomas, el Nanocuerpo o polipéptido específico de la invención a ser usada, la ruta específica de administración y formulación o composición farmacéutica a ser usada, la edad, sexo, peso, dieta, condición general del paciente, y factores similares bien conocidos por el médico.

Generalmente, el régimen de tratamiento comprenderá la administración de uno o más Nanocuerpos y/o

1006

polipéptidos de la invención, o de una o más composiciones que comprende el mismo, en una o más cantidades o dosis farmacéuticamente efectiva. Las cantidades o dosis específicas a ser administradas se pueden determinar por el médico, nuevamente en base a los factores citados anteriormente.

Generalmente, para la prevención y/o tratamiento de las enfermedades y trastornos mencionados en este documento y dependiendo de la enfermedad o trastorno específico a ser tratado, la potencia del Nanocuerpo y polipéptido específico de la invención a ser usada, la ruta específica de administración y la formulación o composición farmacéutica específica usada, los Nanocuerpos y polipéptidos de la invención generalmente serán administrados en una cantidad entre 1 gramo y 0.01 microgramo por kg de peso corporal por día, preferiblemente entre 0.1 gramo y 0.1 microgramo por kg de peso corporal por día, tal como aproximadamente 1, 10, 100 ó 1000 microgramos por kg de peso corporal por día, ya sea continuamente (por ejemplo, infusión), como una dosis diaria única o como múltiples dosis divididas durante el día. El médico generalmente será capaz para determinar una dosis diaria adecuada, dependiendo de los factores mencionados en este documento. También será claro que en casos específicos, el médico puede escoger para desviarse

de estas cantidades, por ejemplo en las bases de los factores citados anteriormente y su juicio experto. Generalmente, algo de orientación en las cantidades a ser administradas se puede obtener de las cantidades usualmente administradas para anticuerpos o fragmento de anticuerpo convencionales comparables contra el mismo objetivo administrado vía esencialmente la misma ruta, tomando en cuenta sin embargo diferencias en afinidad/avidez, eficacia, biodistribución, vida media y factores similares bien conocidos por la persona experta.

Usualmente, en el método anterior, se usará un Nanocuerpo o polipéptido único de la invención. Sin embargo dentro del alcance de la invención se usas dos o más Nanocuerpos y/o polipéptidos de la invención en combinación.

Los Nanocuerpos y polipéptidos de la invención también se pueden usar en combinación con uno o más compuestos o principio farmacéuticamente activos adicionales, es decir, como un régimen de tratamiento combinado, el cual puede o no puede conducir a un efecto sinergístico. Nuevamente, el médico será capaz de seleccionar tales compuestos o principios adicionales, así como un régimen de tratamiento combinado adecuado, en base a los factores citados anteriormente y su juicio experto.

En particular, los Nanocuerpos y polipéptidos de

1008

la invención se puede usar en combinación con otros compuestos o principios farmacéuticamente activos o se pueden usar para la prevención y/o tratamiento de las enfermedades y trastornos citados en este documento, como un resultado del cual se puede o no se puede obtener un efecto sinergístico. Ejemplos de tales compuestos y principios, así como rutas, métodos y formulaciones o composiciones farmacéuticas para administrarlos, después será claro al médico.

Cuando dos o más sustancias o principios se usan como parte de un régimen de tratamiento combinado, se pueden administrar vía la misma ruta de administración o vía diferentes rutas de administración, en esencialmente el mismo tiempo o en tiempos diferentes (por ejemplo, esencialmente de forma simultánea, consecutivamente o de conformidad a un régimen alternativo). Cuando las sustancias o principios se administran simultáneamente vía la misma ruta de administración, se pueden administrar como formulaciones o composiciones farmacéuticas diferentes, o parte de una formulación o composición farmacéutica combinada, será claro a una persona experta.

También, cuando dos o más sustancias o principios activos se usan como parte de un régimen de tratamiento combinado, cada una de las sustancias o principios se pueden administrar en la misma cantidad y de conformidad

1009

con el mismo régimen como se usa cuando el compuesto o principio se usa en sí mismo, y tal combinación usada puede o no puede conducir a un efecto sinergístico. Sin embargo, cuando se usa lo combinado de dos o más sustancias o principios activos conducen a un efecto sinergístico, puede también ser posible reducir la cantidad de una, más o todas las sustancias o principios a ser administrados, mientras se logra la acción terapéutica deseada. Esto puede por ejemplo se usado para evitar, limitar o reducir cualquiera de los efectos colaterales no deseados que están asociados con el uso de una o más de las sustancias o principios cuando se usan en sus cantidades usuales, mientras se obtiene el efecto farmacéutico o terapéutico deseado.

La efectividad del régimen de tratamiento usado de conformidad con la invención, puede ser determinado y/o seguido por cualquier manera conocida per se para la enfermedad o trastorno involucrado, será claro para el médico. El médico también será capaz, en donde es apropiado y, o en bases caso por caso, para cambiar o modificar un régimen de tratamiento particular, para lograr el efecto terapéutico deseado, para evitar, limitar o reducir los efectos colaterales no seseados, y/o para lograr un balance apropiado entre lograr el efecto terapéutico deseado por una parte y evitar, limitar o reducir los efectos colaterales no deseados por otra parte.

Generalmente, el régimen de tratamiento será seguido hasta que se logre el efecto terapéutico deseado y/o tan largo como el efecto terapéutico deseado se mantenga. Nuevamente, esto se puede determinar por el médico.

Así, en n aspecto adicional, la invención se refiere a una composición farmacéutica que contiene al menos un Nanocuerpo de la invención o al menos un polipéptido de la invención, y al menos un portador adecuado (es decir, un portador adecuado para uso veterinario), y opcionalmente una o más sustancias activas adicionales.

La invención también se refiere al uso de un Nanocuerpo de la invención y/o de un polipéptido de la invención en la preparación de una composición farmacéutica, en particular en la preparación de una composición farmacéutica para la prevención y/o tratamiento (que incluye pero no se limita a alivio de al menos un síntoma) de una enfermedad o trastorno mediado por TNF-alfa y/o asociado con TNF-alfa (por ejemplo, asociado con actividad anormal de TNF-alfa, niveles anormales de TNF-alfa, expresión anormal de TNF-alfa y/o sensibilidad o respuesta anormal a TNF-alfa, o de uno fenómeno biológico asociado con TNF-alfa), tal como una de las enfermedades o trastornos mencionados anteriormente.

La invención también se refiere a un método para prevenir y/o tratar (incluye pero no se limita al alivio de al menos un síntoma) de una enfermedad o trastorno mediado por TNF-alfa y/o asociado con TNF-alfa (por ejemplo, asociado con una actividad anormal de TNF-alfa, niveles anormales de TNF-alfa, expresión anormal de TNF-alfa y/o sensibilidad o respuesta anormal a TNF-alfa, o de un fenómeno biológico asociado con TNF-alfa), tal como una de las enfermedades o trastornos mencionados anteriormente, dicho método comprende administrar a un sujeto en necesidad del mismo, una cantidad terapéuticamente activa de un Nanocuerpo de la invención, de polipéptido de la invención y/o de una composición farmacéutica como se describe anteriormente.

La presente invención proporciona polipéptidos que comprenden uno o más anticuerpos dirigidos hacia factor alfa de necrosis de tumor (TNF-alfa). La presente invención además se refiere a su uso en diagnosis y terapia. Tales anticuerpos pueden tener una secuencia de estructura con alta homología a las secuencias de estructura de humano. Se describen composiciones que comprenden anticuerpos para factor alfa de necrosis de tumo (TNF-alfa) solas o en combinación con otros fármacos.

El factor alfa de necrosis de tumor (TNF-alfa) se cree que juega un papel importante en varios trastornos,

por ejemplo, en trastornos inflamatorios tales como artritis reumatoide, enfermedad de Crohn, colitis ulcerativa y esclerosis múltiple. Tanto TNF-alfa como los receptores (CD120a, CD120b) se han estudiado en mayor detalle. El TNF-alfa en su forma bioactiva en un trímero y la ranura formada por subunidades cercanas es importante para la interacción citocina-receptor. Se han desarrollado varias estrategias para antagonizar la acción de la citocina y actualmente se usan para tratar varios estados de enfermedad.

Un inhibidor TNF-alfa el cual tiene suficiente especificidad y selectividad para TNF-alfa puede ser un compuesto farmacéutico terapéutico o profiláctico para prevenir o tratar trastornos en donde TNF-alfa se ha implicado como agente causativo. Se han descrito métodos para tratar choque tóxico (EP 486526), regresión de tumor, inhibición de cotitoxicidad (US 6448380, US 6451983, US 6498237), enfermedad autoinmune tal como RA y enfermedad de Crohn (EP 663836, US 5672347, US 5656272), injerto contra reacción del hospedante (US 5672347), meningitis bacteriana (EP 585705) por medio de un anticuerpo para TNF-alfa.

Aún ninguno de los fármacos actualmente disponibles son completamente efectivos para el tratamiento de enfermedad autoinmune, y más se limitan por toxicidad severa. Además, es extremadamente difícil y un proceso

largo desarrollar una nueva entidad química (NCE) con suficiente potencia y selectividad para tal secuencia objetivo. Terapéuticos a base de anticuerpo por otro lado tienen potencial significante como fármacos porque tiene especificidad exquisita a su objetivo y una toxicidad inherente baja. Además, el tiempo de desarrollo puede ser considerablemente reducido cuando se compara al desarrollo de nuevas entidades químicas (NCE). Sin embargo, se dificultan aumentar anticuerpos convencionales contra proteínas multiméricas en donde el dominio unido al receptor del ligando es incrustado en una ranura, como es el caso con TNF-alfa. Se describen en la invención anticuerpos de cadena pesada los cuales se suministran de Camelidae, se conocen por tener propensión a unión de cavidad (WO97/49805; Lauwereys et al., EMBO J. 17, 5312, 1998)). Por lo tanto, tales anticuerpos de cadena pesada son adecuadamente inherentes para unir dominios de enlace al receptor de tales ligandos como TNF. Además, tales anticuerpos se conocen por ser estables durante largos periodos de tiempo, por lo tanto incrementar su vida media (Perez et al., Biochemistry, 40, 74, 2001). Además, tales fragmentos de anticuerpo de cadena pesada pueden ser producidos "en masa" en fermentadores, usando sistemas de expresión económicos comparados a fermentación de cultivo celular de mamífero, tal como levaduras y otros

microorganismos (EO 0 698 097).

El uso de anticuerpos derivados de fuentes tales como ratón, oveja, cabra, conejo, etc., y humanizados derivados de los mismos como un tratamiento para condiciones las cuales requieren una modulación de inflamación, es problemático por varias razones. Anticuerpos tradicionales no son estables a temperatura ambiente, y pueden ser refrigerados para preparación y almacenaje, que requiere equipamiento de laboratorio refrigerado necesario, almacenaje y transporte, lo cual contribuye hacia tiempo y costo. La refrigeración es algunas veces no factible en países en desarrollo. Además, la manufactura o producción a escala menor de dichos anticuerpos es costoso porque los sistemas celulares de mamífero necesarios para la expresión de anticuerpos intactos y activos requieren altos niveles de soporte en términos de tiempo y equipamiento, y los rendimientos son muy bajos. Además el tamaño grande de anticuerpos convencionales, puede restringir la penetración de tejido, por ejemplo, en el sitio de tejido inflamado. Además, anticuerpos tradicionales tiene una actividad enlazante la cual depende del pH, y por lo tanto son inadecuados para uso en ambientes externos del intervalo de pH fisiológico usual tal como, en hemorragia gástrica, cirugía gástrica. Además, los anticuerpos tradicionales son inestables a pH

menor o mayor y por lo tanto no son adecuados para administración oral. Sin embargo, se ha demostrado que los anticuerpos camelidae resisten condiciones severas, tal como pH extremo, reactivos de desnaturalización y temperaturas altas (Dumoulin et al., Protein Science 11, 500, 2002), para hacerlos adecuados para suministro por administración oral. Además, los anticuerpos tradicionales tienen una actividad enlazante, la cual depende de la temperatura, y por lo tanto son inadecuados para uso en ensayos o kits de funcionamiento a temperaturas fuera de intervalos de temperatura biológicamente activa (por ejemplo, 37 ± 20°C).

Terapéuticos de polipéptido y en particular terapéuticos en base a anticuerpos tienen potencial significante como fármacos porque tienen especificidad exquisita para su objetivo y una toxicidad inherente baja. Sin embargo, se conoce por el experto destinatarios que un anticuerpo, el cual ha sido obtenido para un objetivo terapéuticamente útil requiere modificación adicional para preparar para terapia humana, para evitar una reacción inmunológica no deseada en un individuo humano en la administración de este. El proceso de modificación es comúnmente llamado "humanización". Se conoce por el experto en la técnica que anticuerpos cultivados en especies, diferentes a humanos, requieren humanización para

proporcionar los anticuerpos terapéuticamente útil en humanos ((1) injerto CDR: Proteina Design Labs: US 6180370, US 5693761; Genentech US 6054297; Celltech: 460167, EP 626390, US 5859205; (2) Veneering: Xoma: US 586919, US 5766886, US 5821123). Existe una necesidad para un método para producir anticuerpos, los cuales evitan el requerimiento para humanización sustancial, o los cuales completamente obvian la necesidad para humanización. Existe una necesidad para una nueva clase de anticuerpos, los cuales tienen regiones de estructura definida o residuos de aminoácido y los cuales pueden ser administrados a un sujeto humano sin el requerimiento para humanización sustancial, o la necesidad para humanización completa.

Otra importante desventaja de anticuerpos convencionales es que son complejos, moléculas grandes y por lo tanto relativamente inestables, y son sensibles a rompimiento por proteasa. Esto significa que los fármacos de anticuerpo convencional no pueden ser administrados oralmente, sublingualmente, tópicamente, nasalmente, vaginalmente, rectalmente o por inhalación porque no resisten el pH bajo en estos sitios, la acción de proteasa en estos sitios y en la sangre y/o porque su tamaño es grande. Tienen que ser administrados por inyección (intravenosamente, subcutáneamente, etc.) para superar alguno de estos problemas. La administración por inyección

1017

requiere capacitación especial para uso de una jeringa y aguja hipodérmica correctamente y seguramente. Además se requiere equipamiento estéril, una formulación líquida del polipéptido terapéutico, vía envasado del polipéptido en una forma estéril y estable y, del sujeto, un sitio adecuado para meter la aguja. Además, los sujetos comúnmente experimentan estrés físico y psicológico antes a y después de recibir una inyección. Por lo tanto, existe una necesidad para un método para el suministro de polipéptidos terapéuticos el cual evita la necesidad de inyección la cual no es únicamente ahorro costo/tiempo, pero el cual también es más conveniente y más confortable para el sujeto.

Terapéuticos en base a Nanocuerpos tienen potencial significante como fármacos porque tienen especificidad exquisita para su objetivo y una toxicidad inherente baja. Sin embargo, mejorando además su afinidad intrínseca y funcional puede conducir a muchos beneficios para un paciente, tal como reducir la dosis del terapéutico; terapia más rápida y efectos colaterales reducidos.

Una modalidad de la presente invención es un Nanocuerpos anti-TNF-alfa, dicho nanocuerpos es preferiblemente como además se define anteriormente.

Una modalidad de la presente invención es un

polipéptido anti-TNF-alfa que comprende al menos un anticuerpos anti-TNF-alfa, dicho polipéptido es preferiblemente como además se define anteriormente.

Otra modalidad de la presente invención es un polipéptido anti-TNF-alfa como se describe además anteriormente, que comprende al menos un nanocuerpos directamente contra una proteína de suero.

Otra modalidad de la presente invención es un polipéptido anti-TNF-alfa como se describe anteriormente, en donde la proteína de suero es cualquier albúmina de suero, inmunoglobulinas de suero, proteína que se enlaza a tiroxina, transferrina o fibrógeno.

Otra modalidad de la presente invención es un polipéptido anti-TNF-alfa como se describe anteriormente, además comprende al menos un nanocuerpo seleccionado del grupo que consiste de nanocuerpo anti-IFN-gama, nanocuerpo del receptor anti-TNF-alfa y nanocuerpo del receptor anti-IFN-gama.

Otra modalidad de la presente invención es un polipéptido anti-TNF-alfa como se describe anteriormente, en donde el número de nanocuerpos dirigidos contra TNF-alfa es al menos dos.

Otra modalidad de la presente invención es un polipéptido anti-TNF-alfa como se describe anteriormente, en donde al menos un nanocuerpo es un V_{HH}s Camelidae

humanizado.

Otra modalidad de la presente invención es una composición que comprende un polipéptido anti-TNF-alfa, como se describe anteriormente y al menos un nanocuerpo del grupo que consiste de nanocuerpos anti-IFN-gama, nanocuerpo del receptor anti-TNF-alfa y nanocuerpo del receptor anti-IFN-gama, para simultáneamente, administración separada o secuencial a un sujeto.

Otra modalidad de la presente invención es un polipéptido anti-THF-alfa como se describe anteriormente, o una composición como se describe anteriormente, en donde el nanocuerpo es una secuencia homóloga, una porción funcional o una porción funcional de una secuencia homóloga del nanocuerpo de longitud completa.

Otra modalidad de la presente invención es un polipéptido anti-TNF-alfa como se describe anteriormente, o una composición como se describe anteriormente, en donde el polipéptido anti-TNF-alfa es una secuencia homóloga, una porción funcional, o una porción funcional de una secuencia homóloga del polipéptido anti-TNF-alfa de longitud completa.

Otra modalidad de la presente invención es un polipéptido anti-TNF-alfa como se describe anteriormente en donde al menos un nanocuerpo es un V_{HH} Camelidae.

Otra modalidad de la presente invención es un

1020

ácido nucleico que codifica un polipéptido anti-TNF-alfa como se describe anteriormente.

Otra modalidad de la presente invención es un método para identificar un agente que modula el enlace de un polipéptido anti-TNF-alfa como se describe anteriormente, para Factor alfa de Necrosis del Tumor que comprende las etapas de:

(a) poner en contacto un polipéptido anti-TNF-alfa como se describe anteriormente con un objetivo que es Factor alfa de Necrosis del Tumor, en la presencia y ausencia de un modulador candidato bajo condiciones que permite en enlace entre el polipéptido y objetivo, y

(b) medir el enlace entre el polipéptido y objetivo de la etapa (a), en donde una disminución en el enlace en la presencia del modulador candidato, en relación al enlace en la ausencia del modulador candidato identifica el modulador candidato como un agente que modula el enlace de un polipéptido anti-TNF-alfa como se describe anteriormente y Factor alfa de Necrosis de Tumor.

Otra modalidad de la presente invención es un método para identificar un agente que modula trastornos mediados por el Factor alfa de Necrosis de Tumor a través del enlace de un polipéptido anti-TNF-alfa como se describe anteriormente para Factor alfa de Necrosis del Tumor que comprende:

1021

(a) poner en contacto un polipéptido anti-TNF-alfa como se describe anteriormente con un objetivo que es Factor alfa de Necrosis de Tumor, en la presencia y ausencia de un modulador candidato bajo condiciones que permiten en enlace entre el polipéptido y el objetivo, y

(b) medir el enlace entre el polipéptido y objetivo de la etapa (a), en donde una disminución en el enlace en la presencia del modulador candidato, en relación al enlace en la ausencia del modulador candidato identificado, el modular candidato como un agente que modula trastornos mediados por el Factor alfa de Necrosis de Tumor.

Otra modalidad de la presente invención es un método para identificar un agente que modula el enlace del Factor alfa de Necrosis de Tumor a su receptor, a través del enlace de un polipéptido anti-TNF-alfa como se describe anteriormente para Factor alfa de Necrosis de Tumor que comprende:

(a) poner en contacto un polipéptido anti-TNF-alfa como se describe anteriormente con un objetivo que es Factor alfa de Necrosis de Tumor, en la presencia y ausencia de un modular candidato bajo condiciones que permiten en enlace entre el polipéptido y objetivo, y

(b) medir en enlace entre el polipéptido y objetivo de la etapa (a), en donde disminuye el enlace en

la presencia del modulador candidato, en relación al enlace en la ausencia del modulador candidato identificado como el modulador candidato como un agente que modula el enlace del Factor alfa de Necrosis de Tumor para su receptor.

Otra modalidad de la presente invención es un kit para selección de los agentes que modula trastornos mediados por el Factor alfa de Necrosis de Tumor, que comprende un polipéptido anti-TNF-alfa como se describe anteriormente y Factor alfa de Necrosis de Tumor.

Otra modalidad de la presente invención es un agente desconocido que modula el enlace de un polipéptido anti-TNF-alfa como se describe anteriormente para Factor alfa de Necrosis de Tumor, identificado de conformidad con el método descrito anteriormente.

Otra modalidad de la presente invención es un agente desconocido que modula trastornos mediados por el Factor alfa de Necrosis de Tumor, identificado de conformidad con los métodos como se describen anteriormente.

Otra modalidad de la presente invención es un agente desconocido como se describe anteriormente, en donde los trastornos son una o más inflamaciones, artritis reumatoide, enfermedad de Crohn, colitis ulcerativa, síndrome del intestino inflamatorio y esclerosis múltiple.

Otra modalidad de la presente invención es un

polipéptido anti-TNF-alfa como se describe anteriormente, o un ácido nucleico como se describe anteriormente, o una composición como se describe anteriormente, o un agente como se describe anteriormente para tratar y/o prevenir y/o aliviar trastornos que se relacionan a procesos inflamatorios.

Otra modalidad de la presente invención es un uso de un polipéptido anti-TNF-alfa como se describe anteriormente o un ácido nucleico como se describe anteriormente, o una composición como se describe anteriormente, o un agente como se describe anteriormente para la preparación de un medicamento para tratar y/o prevenir y/o aliviar trastornos que se relacionan a reacciones inflamatorias.

Otra modalidad de la presente invención es un polipéptido anti-TNF-inflamatorio como se describe anteriormente o una composición como se describe anteriormente, para tratar y/o prevenir y/o aliviar trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención, el cual es capaz de pasar a través del ambiente gástrico sin que la sustancia sea activada.

Otra modalidad de la presente invención es un uso de un polipéptido anti-TNF-alfa como se describe anteriormente o una composición como se describe

anteriormente, para la preparación de un medicamento para tratar, prevenir y/o aliviar los síntomas de trastornos susceptibles para modulación por un Nanocuerpo o polipéptido de la invención, el cual es capaz de pasar a través del ambiente gástrico son que la sustancia sea activada.

Otra modalidad de la presente invención es un polipéptido anti-TNF-alfa como se describe anteriormente o una composición como se describe anteriormente, para tratar y/o prevenir y/o aliviar trastornos susceptibles para modulación por un Nanocuerpo o polipéptido de la invención suministrado al tracto vaginal y/o rectal.

Otra modalidad de la presente invención es un uso de un polipéptido anti-TNF-alfa como se describe anteriormente o una composición como se describe anteriormente, para la preparación de un medicamento para tratar, prevenir y/o aliviar los síntomas de trastornos susceptibles para modulación por un Nanocuerpo o polipéptido de la invención suministrado al tracto vaginal o rectal.

Otra modalidad de la presente invención es un polipéptido anti-TNF-alfa como se describe anteriormente o una composición como se describe anteriormente, para tratar y/o prevenir y/o aliviar trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención

suministrado a la nariz, tracto respiratorio superior y/o pulmón.

Otra modalidad de la presente invención es un uso de un polipéptido anti-TNF-alfa como de describe anteriormente o una composición como se describe anteriormente, para la preparación de un medicamento para tratar, prevenir y/o aliviar los síntomas de trastornos susceptibles para modulación por un Nanocuerpo o polipéptido de la invención suministrado a la nariz, tracto respiratorio superior y/o pulmón.

Otra modalidad de la presente invención es un polipéptido anti-TNF-alfa como se describe anteriormente o una composición como se describe anteriormente, para tratar y/o prevenir y/o alivio de trastornos susceptibles para modulación por un Nanocuerpo o polipéptido de la invención que se suministra a la mucosa intestinal, en donde el trastorno incrementa la permeabilidad de la mucosa intestinal.

Otra modalidad de la presente invención es un uso de un polipéptido anti-TNF-alfa como se describe anteriormente o una composición como se describe anteriormente, para la preparación de un medicamento para tratar, prevenir y/o aliviar los síntomas de trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención que se suministra a la mucosa intestinal,

en donde el trastorno incrementa la permeabilidad de la mucosa intestinal.

Otra modalidad de la presente invención es un polipéptido anti-TNF-alfa como se describe anteriormente o una composición como se describe anteriormente, para tratar y/o prevenir y/o aliviar trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención el cual es capaz de pasar a través de los tejidos por atrás de la lengua efectivamente.

Otra modalidad de la presente invención es un uso de un polipéptido anti-TNF-alfa como se describe anteriormente o una composición como se describe anteriormente, para la preparación de un medicamento para tratar, prevenir y/o aliviar los síntomas de trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención la cual es capaz a través de los tejidos por atrás de la lengua efectivamente.

Otra modalidad de la presente invención es un polipéptido anti-TNF-alfa como se describe anteriormente o una composición como se describe anteriormente, para tratar y/o prevenir y/o aliviar trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención, el cual es capaz de pasa a través de la piel efectivamente.

Otra modalidad de la presente invención es un uso de un polipéptido anti-TNF-alfa como se describe

anteriormente o una composición como se describe anteriormente, para la preparación de un medicamento para tratar, prevenir y/o aliviar los síntomas de trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención, el cual es capaz de pasar a través de la piel efectivamente.

Otra modalidad de la presente invención es un método como se describe anteriormente, un kit como se describe anteriormente, un ácido nucleico o agente como se describe anteriormente, uso de un ácido nucleico o agente como se describe anteriormente, una composición como se describe anteriormente, uso de una composición como se describe anteriormente, un polipéptido anti-TNF-alfa como se describe anteriormente, uso de un polipéptido anti-TNF-alfa como se describe anteriormente en donde los trastornos son cualquiera de inflamación, artritis reumatoide, COPD, asma, enfermedad de Crohn, colitis ulcerativa, síndrome del intestino inflamatorio, esclerosis múltiple, enfermedad de Addison, hepatitis autoinmune, parotitis autoinmune, Diabetes Tipo I, Epididimitis, Glomerulonefritis, enfermedad de Graves, síndrome de Guillain-Barre, enfermedad de Hashimoto, anemia Hemolítica, Lupus eritematoso sistémico, infertilidad del macho, esclerosis múltiple, Myasthenia Gravis, Pénfigos, Psoriasis, fiebre reumática, artritis reumática, Sarcoiditis, Escleroderma,

síndrome de Sjogren, Espondilo artropatías, Tiroiditis y Vasculitis.

Otra modalidad de la presente invención es una composición que comprende un ácido nucleico o agente como se describe anteriormente, un polipéptido anti-TNF-alfa como se describe anteriormente, o una composición como se describe anteriormente, y un vehículo farmacéutico adecuado.

Otra modalidad de la presente invención es un método para diagnosticar un trastorno caracterizado por la disfunción del Factor alfa de Necrosis de Tumor que comprende:

(a) poner en contacto una muestra con un polipéptido anti-TNF-alfa como se describe anteriormente,

(b) detectar un enlace del polipéptido a la muestra; y

(c) comparar el enlace detectado en la etapa (b) con un estándar, en donde una diferencia en enlace relativo a la muestra es diagnóstico de un trastorno caracterizado por disfunción del Factor alfa de Necrosis de Tumor.

Otra modalidad de la presente invención es un kit para seleccionar un trastorno como se cita anteriormente, usando un método como se describe anteriormente.

Otra modalidad de la presente invención es un kit para seleccionar un trastorno como se cita anteriormente,

que comprende un polipéptido anti-TNF-alfa aislado como se describe anteriormente.

Otra modalidad de la presente invención es un uso de un polipéptido anti-TNF-alfa como se describe anteriormente para la purificación del Factor alfa de Necrosis de Tumor.

Otra modalidad de la presente invención es un uso de un polipéptido anti-TNF-alfa como se describe anteriormente para inhibir la interacción entre el Factor alfa de Necrosis de Tumor y uno o más receptores del Factor alfa de Necrosis de Tumor.

Otra modalidad de la presente invención es un método para producir un polipéptido anti-TNF-alfa como se describe anteriormente, que comprende las etapas de:

(a) obtener un ADN de ramificación doble que codifica un V_{HH} Camelidae dirigido al Factor alfa de Necrosis de Tumor,

(b) clonar y expresar el ADN seleccionado en la etapa (b).

Otra modalidad de la presente invención es un método para producir un polipéptido anti-TNF-alfa como se describe anteriormente, que comprende:

(a) cultivar las células hospedantes, que comprende ácido nucleico capaz de codificar un polipéptido anti-TNF-alfa como se describe anteriormente, bajo

condiciones que permite la expresión del polipéptido, y,

(b) remover el polipéptido producido del cultivo.

Otra modalidad de la presente invención es un método como se describe anteriormente, en donde las células hospedantes son bacterias y levaduras.

Otra modalidad de la presente invención es un kit para seleccionar cualquiera de inflamación, artritis reumatoide, enfermedad de Crohn, colitis ulcerativa, síndrome del intestino inflamatorio o esclerosis múltiple que comprende un polipéptido anti-TNF-alfa como se describe anteriormente.

V_{HH}s, de conformidad con la presente invención, y como se conoce por destinatario experto son dominios variables de cadena pesada derivados de inmunoglobulinas que carecen naturalmente de cadenas ligeras tales como aquellas derivadas de Camelidae como se describe en el documento WO 94/04678 (y referido en adelante como dominios V_{HH} o nanocuerpos). Las moléculas V_{HH} son aproximadamente 10x más pequeñas que las moléculas IgG. Son polipéptidos únicos y muy estables, que resisten pH y condiciones de temperatura extremas. Sin embargo, son resistentes a la acción de proteasas lo cual no es el caso para anticuerpos convencionales. Además, la expresión in vitro de V_{HH}s produce alto rendimiento, V_{HH}s funcional apropiadamente plegada. Además, anticuerpos generados en Camélidos

reconocerán epítopes diferentes de aquellos reconocidos por anticuerpos generados in vitro a través del uso de bibliotecas de anticuerpo vía inmunización de mamífero diferentes de Camélidos (WO 9749805). Tal como, V_{HH}s anti-TNF-alfa puede interactuar más eficientemente con TNF-alfa que anticuerpos convencionales, por lo tanto bloquea su interacción con el receptor TNF-alfa más eficientemente.

El TNF-alfa es también un fragmento de TNF-alfa, capaz de provocar una respuesta inmune. El TNF-alfa es también un fragmento de TNF-alfa, capaz de enlazare a un nanocuerpo mejorado contra el TNF-alfa de longitud completa.

Un nanocuerpo dirigido contra TNF-alfa significa nanocuerpo que es capaz de enlazarse a TNF-alfa con una afinidad de superior que 10^{-6}M.

Una modalidad de la presente invención es un polipéptido anti-TNF, en donde los nanocuerpos comprenden V_{HH} Camelidae dirigido contra TNF-alfa.

Uno o más anticuerpos del polipéptido anti-TNF los cuales son dirigidos contra un TNF-alfa puede ser de la misma secuencia. Alternativamente no todos pueden tener la misma secuencia. Dentro del alcance de la invención que un polipéptido anti-TNF comprende nanocuerpos anti-TNF-alfa las cuales no todos comparten la misma secuencia, pero las cuales se dirigen contra el mismo objetivo, uno o más

1032

antígenos del mismo.

La presente invención además se refiere a un polipéptido anti-TNF-alfa, en donde el nanocuerpo es un V_{HH} dirigido contra TNF-alfa, en donde el V_{HH} pertenece a una clase que tiene secuencias similares a la humana. Las clases se caracterizan porque V_{HH} porta un aminoácido a partir del grupo que consiste de glicona, alanina, valina, leucina, isoleucina, prolina, fenilalanina, tirosina, triptofano, metionina, serina, treonina, aspargina o glutamina en posición 45, tal como, por ejemplo, L45 y un triptofano en posición 103, de conformidad con la numeración Kabat. Otra clase similar a la humana de nanocuerpos Camelidae se han descrito en el documento WO03035696 y contiene los residuos FR2 hidrofóbicos típicamente encontrados en anticuerpos convencionales de origen humano o de otras especies, pero compensando esta pérdida en hidrofilicidad por el residuo de arginina cargada en posición 103, que sustituye el residuo de triptofano conservado presente en VH de anticuerpos de cadena doble. Como tal, los péptidos que pertenecen a estas dos clases muestran una homología de secuencia de aminoácido alta a regiones de estructura VH humana y los péptidos pueden ser administrados a un humano directamente sin expectativa de una respuesta inmune indeseada de ahí, y sin la carga de humanización adicional. La invención

también se refiere a ácidos nucleicos capaces de codificar los polipéptidos.

Cualquiera de los V_{HH} como se usan por la invención pueden ser de la clase tradicional o de la clase de anticuerpos Camelidae similar a humanos. Los anticuerpos se pueden dirigir contra TNF-alfa completo o un fragmento del mismo, o un fragmento de una secuencia homologa del mismo. Estos polipéptidos incluyen los anticuerpos Camelidae de longitud completa, llamados dominios Fc y V_{HH}, versiones quiméricas de anticuerpos Camelidae de cadena pesada con un dominio Fc humano o V_{HH} por si mismos o fragmentos derivados.

Los V_{HH} de albúmina anti-suero pueden interactuar en una manera más eficiente con albúmina de suero que anticuerpos convencionales los cuales se conoce por ser una proteína portadora. Como una proteína portadora algo de los epítopes de albúmina de suero pueden ser inaccesibles por proteínas enlazadas, péptidos y compuestos químicos pequeños. Ya que se conocen V_{HH} por unirse a epítopes "inusuales" o no convencionales tales como cavidades (WO 97/49805), la afinidad de tal V_{HH} que hace circular albúmina puede ser incrementada.

La presente invención se refiere al descubrimiento que un polipéptido anti-TNF como se describe en este documento, además comprende uno o más anticuerpos

dirigidos contra una o más proteínas de suero de un sujeto, sorprendentemente tiene vida media prolongada en la circulación del sujeto, comparado con la vida media del nanocuerpo anti-TNF-alfa cuando no parte del constructo. Además, los polipéptidos se encuentran por exhibir las mismas propiedades favorables de nanocuerpos tal como alta estabilidad que permanece intacta en ratones, resistencia a pH extremo, estabilidad a alta temperatura y alta afinidad de objetivo.

La proteína de suero puede ser cualquier proteína adecuada encontrada en el suero del sujeto. En un aspecto de la invención, la proteína de suero es albúmina de suero, inmunoglobulinas de suero, proteínas que se enlazan a tiroxina, transferrina o fibrinógeno. Dependiendo del uso planeado, tal como la vida media requerida para tratamiento efectivo y/o formación en compartimiento del antígeno objetivo, el precursor V_{HH} se puede dirigir a una de las proteínas de suero anteriores.

De acuerdo a un aspecto específico, pero no limitante de la invención, el Nanocuerpo contra albúmina de suero humano que consiste de 4 regiones de estructura (FR1 a FR4 respectivamente) y 3 regiones determinantes complementarias (CDR1 a CDR3 respectivamente), en el cual:

(iv) CDR1 es una secuencia de aminoácido seleccionada del grupo que consiste de:

SFGMS	[SEC ID NO: 36]
LNLMG	[SEC ID NO: 37]
INLLG	[SEC ID NO: 38]
NYWMY;	[SEC ID NO: 39]

y/o del grupo que consiste de secuencias de aminoácido que tienen 2 o únicamente 1 "diferencias de aminoácido" (como se define en este documento) con una de las secuencias de aminoácido anteriores en la cual:

(1) cualquiera de las sustituciones de aminoácido es preferiblemente una sustitución de aminoácido conservativo (como se define en este documento) y/o

(2) la secuencia de aminoácido preferiblemente únicamente consiste de sustituciones de aminoácido, y sin supresiones o inserciones de aminoácido, comparado a las secuencias de aminoácido anterior;

y en la cual:

(v) CDR2 es una secuencia de aminoácido escogida del grupo que consiste de:

1036

SISGSGSDTLYADSVKG	[SEC ID NO: 40]
TITVGDSTNYADSVKG	[SEC ID NO: 41]
TITVGDSTSYADSVKG	[SEC ID NO: 42]
SINGRGDDTRYADSVKG	[SEC ID NO: 43]
AISADSSTKNYADSVKG	[SEC ID NO: 44]
AISADSSDKRYADSVKG	[SEC ID NO: 45]
RISTGGGYSYYADSVKG	[SEC ID NO: 46]

o el grupo que consiste secuencias de aminoácido que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99%, identidad de secuencia (como se define en este documento) con una de las secuencias de aminoácido anteriores; en las cuales:

(1) cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativo (como se define en este documento); y/o

(2) secuencia de aminoácido preferiblemente únicamente contiene sustituciones de aminoácido, y sin supresiones o inserciones de aminoácido, comparado a las secuencias de aminoácido anteriores;

y/o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o únicamente 1 "diferencias de aminoácido" (como se define anteriormente) con una de las secuencias de aminoácido anteriores, en las cuales:

1037

(1) cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativo (como se define en este documento); y/o

(2) la secuencia de aminoácido preferiblemente únicamente contiene sustituciones de aminoácido, y sin supresiones o inserciones de aminoácido, comparado a las secuencias de aminoácidos anteriores;

y en la cual:

(iv) CDR3 es una secuencia de aminoácido escogida del grupo que consiste de:

DREAQVDTLDFDY [SEC ID NO: 47]

o del grupo que consiste de secuencias de aminoácido que tienen al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en este documento) con una de las secuencias de aminoácido anteriores; en las cuales

(1) cualquier sustitución es preferiblemente una sustitución de aminoácido conservativo (como se define en este documento) y/o

(2) la secuencia de aminoácido preferiblemente únicamente contiene sustituciones de aminoácido, y sin supresiones o inserciones

1038

de aminoácido, comparado a las secuencias de aminoácido anteriores;

y/o del grupo que consiste de secuencias de aminoácido que tiene 3, 2 o únicamente 1 "diferencias de aminoácido" (como se define anteriormente) con una de las secuencias de aminoácido anteriores, en las cuales:

(1) cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativo (como se define en este documento) y/o

(2) la secuencia de aminoácido preferiblemente únicamente contiene sustituciones de aminoácido, y sin supresiones o inserciones de aminoácido, comparado a las secuencias de aminoácido anteriores;

o del grupo que consiste de:

GGSLSR	[SEQ ID NO: 48]
RRTWHSEL	[SEQ ID NO: 49]
GRSVSRS	[SEQ ID NO: 50]
GRGSP	[SEQ ID NO: 51]

y/o del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o únicamente 1 "diferencia de aminoácido" (como se define en este documento) con una de las secuencias de amino ácido anteriores, en las cuales:

(1) cualquier sustitución de aminoácido es

preferiblemente una sustitución de aminoácido conservativo (como se define en este documento); y/o

(2) la secuencia de aminoácido preferiblemente únicamente contiene sustituciones de aminoácido, y sin supresiones o inserciones de aminoácido, comparado a las secuencias de aminoácido anteriores.

En otro aspecto, la invención se refiere a un Nanocuerpo contra albúmina de suero humano, el cual consiste de 4 regiones de estructura (FR1 a FR4 respectivamente) y 3 regiones determinantes complementarias (CDR1 a CDR3 respectivamente), la cual se escoge del grupo que consiste de anticuerpos de dominio y/o anticuerpos de dominio únicos con una de las siguientes combinaciones de CDR1, CDR2 y CDR3, respectivamente:

- **CDR1:** SFGMS; **CDR2:** SISGSGSDTLYADSVKG; **CDR3:** GGSLSR;
- **CDR1:** LNLMG; **CDR2:** TITVGDSTNYADSVKG; **CDR3:** RRTWHSEL;
- **CDR1:** INLLG; **CDR2:** TITVGDSTSYADSVKG; **CDR3:** RRTWHSEL;
- **CDR1:** SFGMS; **CDR2:** SINGRGDDTRYADSVKG; **CDR3:** GRSVSRS;
- **CDR1:** SFGMS; **CDR2:** AISADSSDKRYADSVKG; **CDR3:** GRGSP;
- **CDR1:** SFGMS; **CDR2:** AISADSSDKRYADSVKG; **CDR3:** GRGSP;
- **CDR1:** NYWMY; **CDR2:** RISTGGGYSYYADSVKG; **CDR3:** DREAQVDTLDFDY.

En los nanocuerpos de la invención que comprende las combinaciones de CDR mencionados anteriormente, cada CDR puede ser reemplazado por un CDR escogido del grupo que

1000

consiste de secuencias de aminoácido que tienen al menos 80%, preferiblemente 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia (como se define en este documento) con los CDR mencionados, en los cuales

(1) cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativo (como se define en este documento); y/o

(2) la secuencia de aminoácido preferiblemente únicamente contiene sustituciones de aminoácido, y sin supresiones o inserciones de aminoácido, comparado a las secuencias de aminoácido anteriores;

y/o escogidos del grupo que consiste de secuencias de aminoácido que tienen 3, 2 o únicamente 1 (como se indica en el párrafo precedente) "diferencias de aminoácido" (como se define en este documento) con los CDR mencionados, una de las secuencias de aminoácido anteriores; en las cuales:

(1) cualquiera de las sustituciones de aminoácido es preferiblemente una sustitución de aminoácido conservativo (como se define en este documento); y/o

(2) la secuencia de aminoácido preferiblemente únicamente contiene sustituciones de

aminoácido, y sin supresiones o inserciones de aminoácido, comparado a las secuencias de aminoácido anteriores.

Sin embargo, de los Nanocuerpos de la invención que comprenden las combinaciones de CDR mencionados anteriormente, Nanocuerpos que comprenden uno o más CDR listados anteriormente son particularmente preferidos; Nanocuerpos que comprenden dos o más de los CDR listados anteriormente son más particularmente preferidos; y Nanocuerpos que comprenden tres de los CDR listados anteriormente son más particularmente preferidos.

En estos Nanocuerpos contra albúmina de suero humano, las regiones de estructura FR1 a FR4 son preferiblemente como se define anteriormente para los Nanocuerpos de la invención.

Particularmente Nanocuerpos preferidos contra albúmina de suero humano se seleccionan de grupos que consisten de SEC ID NOs: 61 a 67, SEC ID NOs: 87 a 89 y SEC ID NOs: 100-104. Las combinaciones preferidas de CDR y regiones de estructura presentes en estos Nanocuerpos también se listan en la Tabla II.

Tabla II. Combinación preferida de secuencias de estructura y CDR en Nanocuerpos contra albúmina de suero humano

Clone	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
PMP6A8 (ALB2)	36	AVQLVESGGGLVQGGG SLRLACAASERIFD	37	LNLMG	38	WYRQGPGN ERELVA	39	TCITVGDSTNYA DSVKG	39	RFTISMDYTKQTVYLHMN SLRPEDTGLYYCKI	40	RRTWHSEL	41	WGQGTQV TVSS
PMP6B4	36	EVQLVESGGGLVQEGG SLRLACAASERIWD	37	INLLG	38	WYRQGPGN ERELVA	39	TITVGDSTSYAD SVKG	39	RFTISRDYDKNTLYLQMN SLRPEDTGLYYCKI	40	RRTWHSEL	41	WGQGTQV TVSS
PMP6A6 (ALB1)	37	AVQLVESGGGLVQPGN SLRLSCAASGFTFR	37	SFGMS	38	WVRQAPGK EPEWVS	39	SISGSGSDTLYA DSVKG	39	RFTISRDNAKTTLYLQMN SLKPEDTAVYYCTI	40	GGSLSR	41	SSQGTQV TVSS
PMP6C1	37	AVQLVDSGGGLVQPGG SLRLSCAASGFSFG	37	SFGMS	38	WVRQYPGK EPEWVS	39	SINGRGDDTRYA DSVKG	39	RFSISRDNAKNTLYLQMN SLKPEDTAEYYCTI	40	GRSVSRS	41	RTQGTQV TVSS
PMP6G8	37	AVQLVESGGGLVQPGG SLRLTCTASGFTFR	37	SFGMS	38	WVRQAPGK DOEWVS	39	AISADSSTKNYA DSVKG	40	RFTISRDNAKKMLYLEMN SLKPEDTAVYYCVI	40	GRGSP	41	SSPGTQV TVSS
PMP6A5	37	QVQLAESGGGLVQPGG SLRLTCTASGFTFG	38	SFGMS	38	WVRQAPGE GLEWVS	39	AISADSSDKRYA DSVKG	40	RFTISRDNAKKMLYLEMN SLKSEDTAVYYCVI	40	GRGSP	41	ASQGTQV TVSS
PMP6G7	37	QVQLVESGGGLVQPGG SLRLSCAASGFTFS	38	NYWMY	38	WVRVAPGK GLERIS	39	RDISTGGGYSYY ADSVKG	40	RFTISRDNAKNTLYLQMN SLKPEDTALYYCAK	40	DREAQVDTLD FDY	41	RGQGTQV TVSS

Otro aspecto de la invención es un polipéptido anti-TNF-alfa como se describe en este documento, además que comprende al menos un polipéptido seleccionado del grupo que consiste de un polipéptido anti-IFN-gama, un polipéptido del receptor anti-TNF-alfa y polipéptido del receptor anti-IFN-gama.

De acuerdo a un aspecto de la invención, un nanocuerpo se dirige contra el receptor TNF-alfa. El nanocuerpo puede ser un V_{HH} Camelidae.

De acuerdo a un aspecto de la invención, un nanocuerpo se dirige contra el receptor IFN-gama. El nanocuerpos puede ser un V_{HH} Camelidae.

Otro aspecto de la invención es un método para tratar una enfermedad autoinmune o condición como se cita en este documento, que comprende administrar a un paciente una cantidad efectiva de un polipéptido anti-TNF-alfa, además comprende al menos un polipéptido seleccionado del grupo que consiste de un polipéptido anti-IFN-gama, polipéptido del receptor anti-TNF-gama y polipéptido del receptor anti-IFN-gama, tal como polipéptidos unidos entre sí como se describe abajo.

Tales constructos multi-específicos pueden tener potencia mejorada como compuesto terapéutico inflamatorio sobre constructos mono-específicos.

Un aspecto de la invención es una composición que

comprende un polipéptido anti-TNF-alfa como se describe en este documento y al menos un polipéptido se selecciona del grupo que consiste de polipéptido anti-IFN-gama, polipéptido del receptor anti-TNF-alfa y polipéptido del receptor anti-IFN-gama, para administración simultánea, separada o secuencia a un sujeto.

Un aspecto de la invención es un método para tratar una enfermedad autoinmune que comprende administrar a un individuo, una cantidad efectiva de un polipéptido anti-TNF-alfa y al menos un polipéptido seleccionado del grupo que consiste de polipéptido anti-IFN-gama, polipéptido del receptor anti-TNF-alfa y polipéptido del receptor anti-IFN-gama, simultáneamente, separadamente o secuencialmente.

Otro aspecto de la invención es un kit que comprende un polipéptido anti-TNF-alfa y al menos un polipéptido seleccionado del grupo que consiste de polipéptido anti-IFN-gama, polipéptido del receptor anti-TNF-alfa y polipéptido del receptor anti-IFN-gama para administración simultánea, separada o secuencia a un sujeto. Es un aspecto de la invención que el kit se puede usar de conformidad con la invención. Es un aspecto de la invención que el kit se puede usar para tratar las enfermedades como se cita en este documento.

Por administración simultánea significa que los

polipéptidos se administran a un sujeto al mismo tiempo. Por ejemplo, como una mezcla de los polipéptidos o una composición que comprende los polipéptidos. Por ejemplo, como una mezcla de los polipéptidos o una composición que comprende los polipéptidos. Ejemplos incluyen, pero no se limitan a una solución administrada intravenosamente, una tableta, líquido, crema tópica, etc., en donde cada preparación comprende las propiedades de interés.

Por administración separada significa que los polipéptidos se administran a un sujeto al mismo tiempo o sustancialmente al mismo tiempo. Los polipéptidos están presentes en el kit como preparaciones no mezcladas, separadas. Por ejemplo, los polipéptidos diferentes pueden estar presentes en el kit como tabletas individuales. Las tabletas pueden ser administradas al sujeto tragando ambas tabletas al mismo tiempo, o una tableta directamente después de la otra.

Por administración secuencial significa que los polipéptidos se administran a un sujeto secuencialmente. Los polipéptidos están presentes en el kit como preparaciones separadas, no mezcladas. Existe un tiempo de intervalo entre dosis. Por ejemplo, un polipéptido puede ser administrado hasta 336, 312, 288, 264, 240, 216, 192, 168, 144, 120, 96, 72, 48, 24, 20, 16, 12, 8, 4, 2, 1 ó 0.5 horas después del otro componente.

En administración secuencial, un polipéptido puede ser administrado una vez, o cualquier número de veces y en varias dosis antes y/o después de la administración de otro polipéptido. La administración secuencial se puede combinar con administración simultánea o secuencial.

Los usos médicos de un polipéptido anti-TNF-alfa descrito abajo, también aplican a la composición que comprende un polipéptido anti-TNF-alfa como se describe en este documento y al menos un polipéptido seleccionado del grupo que consiste de polipéptido anti-IFN-gama, polipéptido del receptor anti-THF-alfa y polipéptido del receptor anti-IFN-gama, para administración simultánea, separada o secuencial a un sujeto como se describe en este documento anteriormente.

De acuerdo a un aspecto de la invención, un polipéptido anti-IFN-gama, un nanocuerpo anti-TNF-gama se dirige contra IFN-gama. El nanocuerpo puede ser un V_{HH} Camelidae.

De acuerdo a un aspecto de la invención, un nanocuerpo anti-TNF-alfa se dirige contra el receptor TNF-alfa. El nanocuerpo puede ser un V_{HH} Camelidae.

De acuerdo a un aspecto de la invención, un polipéptido del receptor anti-IFN-gama, un nanocuerpo anti-TNF-alfa se dirige contra el receptor IFN-gama. El nanocuerpo puede ser un V_{HH} Camelidae.

1047

Otra modalidad de la presente invención es un polipéptido anti-TNF-alfa como se describe en este documento, en donde el número de nanocuerpos que se dirige contra THF-alfa es dos o más. Tales polipéptidos anti-TNF-alfa multivalentes tienen la ventaja de afinidad funcional inusualmente alta para el objetivo, exhibiendo mucho mayores propiedades inhibitorias que las esperadas compararas a su contraparte monovalente.

Los polipéptidos anti-TNF-alfa multivalentes tienen afinidades funcionales que son varias órdenes de magnitudes mayores que los polipéptidos anti-TNF-alfa originales monovalentes. Se ha encontrado que las afinidades funcionales de estos polipéptidos anti-TNF-alfa son mucho mayores que aquellos reportados en la técnica anterior para anticuerpos bivalente o multivalente. Sorprendentemente, los polipéptidos anti-TNF-alfa de la presente invención enlazados entre sí directamente o vía una secuencia enlazadora corta, muestran las afinidades funcionales altas esperadas teóricamente con anticuerpos de cuatro cadenas convencionales multivalentes.

Se ha encontrado que tales actividades funcionales muy incrementadas pueden ser detectadas preferiblemente con antígenos compuestos de proteínas multidominio y multiméricas, ya sea en ensayos de enlace lineal o en ensayos funcionales, por ejemplo, ensayos de

citotoxicidad.

Los nanocuerpos pueden estar unidos para formar cualquiera de los polipéptidos descritos en este documento, que comprenden más de uno de los anticuerpos usando métodos conocidos en la técnica o cualquier método futuro. Por ejemplo, se pueden fusionar por reticulación química haciendo reaccionar residuos de aminoácido con un agente de derivación orgánico tal como se describe por Blattler et al., Biochemistry 24, 1517-1524; EP294703. Alternativamente, el nanocuerpos puede ser fusionado genéticamente en el nivel ADN, es decir, un constructo de polinucleótido formado el cual codifica el constructo de polipéptido completo, que comprende uno o más nanocuerpos anti-objetivo y uno o más nanocuerpos de proteína anti-suero. Un método para reducir un constructo de polipéptido V_{HH} multivalente o bivalente se describe en la solicitud de patente PCT WO 96/34103. Un método de nanocuerpos múltiples unidos es vía la ruta genética enlazando secuencias que codifican nanocuerpos ya sea directamente o por un enlazador de péptido. Por ejemplo, el extremo C-terminal del primer nanocuerpos puede ser enlazado un extremo N-terminal del nanocuerpo siguiente. Esta forma enlazada se puede extender para enlazar nanocuerpos adicionales para la construcción y producción de tri-, tetra-, etc., constructos funcionales.

De acuerdo a un aspecto de la presente invención, los nanocuerpos están enlazados entre sí directamente, sin uso de un enlazador. Contrario a anticuerpos convencionales voluminosos unidos en donde una secuencia enlazadora se necesita retener actividad enlazante en las dos subunidades, polipéptidos de la invención pueden ser directamente enlazados, así evitando los problemas potenciales de la secuencia enlazadora, tal como antigenicidad cuando se administra a un sujeto humano, inestabilidad de la secuencia enlazadora que conduce a disociación de las subunidades.

De acuerdo a otro aspecto de la presente invención, los nanocuerpos se enlazan entre sí vía una secuencia enlazadora del péptido. Tal secuencia enlazadora puede ser una secuencia que se origina de forma natural o una secuencia que se origina de forma no natural. La secuencia enlazadora se espera ser no inmunogénica en el sujeto al cual se administra el polipéptido anti-TNF-alfa. La secuencia enlazadora puede proporcionar suficiente flexibilidad al polipéptido anti-TNF-alfa, al mismo tiempo es resistente a degradación proteólica. Un ejemplo no limitante de una secuencia enlazadora es uno que se puede derivar de la región articulada de V_{HH} descritos en el documento 96/34103.

De acuerdo a otro aspecto de la presente

1070

invención, nanocuerpos multivalentes que comprenden más de dos nanocuerpos, pueden estar enlazados entre sí directamente o vía una secuencia enlazadora. Tales constructos son difíciles para producir con anticuerpos convencionales y debido a un obstáculo estérico de las subunidades voluminosas, funcionalmente pueden ser olvidadas o enormemente disminuidas más que incrementado considerablemente como se observa con V_{HH} de la invención comparado al constructo monovalente.

Los constructos de polipéptido descritos en este documento, se pueden elaborar por el experto en la técnica de conformidad con métodos conocidos en la técnica o cualquier método futuro. Por ejemplo, V_{HH} pueden ser obtenidos usando métodos conocidos en la técnica tal como inmunizando un canal y obtener hibridomas de esto, o clonando una biblioteca de nanocuerpos usando técnicas de biología molecular conocidas en la técnica y selección subsecuente usando exhibición del fago.

De acuerdo a un aspecto de la invención, un polipéptido anti-TNF-alfa puede ser una secuencia homóloga de un polipéptido anti-TNF-alfa de longitud completa. De acuerdo a otro aspecto de la invención, un polipéptido anti-TNF-alfa puede ser una porción funcional de un polipéptido anti-TNF-alfa de longitud completa. De acuerdo a otro aspecto de la invención, un polipéptido anti-TNF-

alfa puede ser una secuencia homóloga de un polipéptido anti-TNF-alfa de longitud completa. De acuerdo a otro aspecto de la invención, un polipéptido anti-TNF-alfa puede ser una porción funcional de una secuencia homóloga de un polipéptido anti-TNF-alfa de longitud completa. De acuerdo a un aspecto de la invención, un polipéptido anti-TNF-alfa puede comprender una secuencia de un polipéptido anti-TNF-alfa.

De acuerdo a un aspecto de la invención, un nanocuerpo usado para formar un polipéptido anti-TNF-alfa puede ser un nanocuerpo completo (por ejemplo, un V_{HH}) o una secuencia homóloga del mismo. De acuerdo a otro aspecto de la invención, un nanocuerpo usado para formar el constructo de polipéptido puede ser una porción funcional de un nanocuerpo completo. De acuerdo a otro aspecto de la invención, un nanocuerpo usado para formar el constructo de polipéptido puede ser una secuencia homóloga de un nanocuerpo completo. De acuerdo a otro aspecto de la invención, un nanocuerpo usado para formar el constructo de polipéptido puede ser una porción funcional de una secuencia homóloga de un nanocuerpo completo.

Como se usa en este documento, una secuencia homóloga de la presente invención, puede comprender adiciones, supresiones o sustituciones de uno o más aminoácidos, los cuales no son sustancialmente alterados de

las características funcionales de los polipéptidos de la invención. El número de supresiones o sustituciones de aminoácido es preferiblemente hasta 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69 ó 70 aminoácidos.

Una secuencia homóloga de conformidad con la presente invención puede ser un polipéptido modificado por la adición, supresión, o sustitución de aminoácidos, tal modificación sustancialmente no altera las características funcionales compradas con el polipéptido no modificado.

Una secuencia homóloga de conformidad con la presente invención, puede comprender un polipéptido modificado por la adición, supresión o sustitución de aminoácidos, tal modificación sustancialmente no altera las características funcionales comparadas con el polipéptido no modificado.

Una secuencia homóloga de conformidad con la presente invención, puede ser una secuencia la cual existe en otras especies de Camelidae tal como, por ejemplo, camello, dromedario, llama, alpaca, guanaco, etc.

En donde la secuencia homóloga indica identidad de secuencia, significa una secuencia la cual presenta una

identidad de secuencia mayor (más de 70%, 75%, 80%, 85%, 90%, 95% o 98% de identidad de secuencia) con la secuencia original y es preferiblemente caracterizada por propiedades similares de la secuencia original, concretamente afinidad, la identidad calculada usando métodos conocidos.

Alternativamente, una secuencia homóloga también puede ser cualquier secuencia de aminoácido que resulta de las sustituciones permitidas en cualquier número de posiciones de la secuencia original de conformidad con la fórmula de abajo:

Ser sustituida por Ser, Thr, Gly y Asn;

Arg sustituida por uno de Arg, His, Gln, Lys y Glu;

Leu sustituida por uno de Leu, Ile, Phe, Tyr, Met y Val;

Pro sustituida por uno de Pro, Gly, Ala y Thr;

Thr sustituida por uno de Thr, Pro, Ser, Ala, Gly, His y Gln;

Ala sustituida por uno de Ala, Gly, Thr y Pro;

Val sustituida por uno de Val, Met, Tyr, Phe, Ile y Leu;

Gly sustituida por uno de Gly, Ala, Thr, Pro y Ser;

Ile sustituida por uno de Ile, Met, Tyr, Phe, Val y Leu;

Phe sustituida por uno de Phe, Trp, Met, Tyr, Ile, Val y Leu;

Tyr sustituida por uno de Tyr, Trp, Met, Phe, Ile, Val y Leu;

His sustituida por uno de His, Glu, Lys, Gln, Thr y Arg;

Gln sustituida por uno de Gln, Glu, Lys, Asn, His, Thr y Arg;

Asn sustituida por uno de Asn, Glu, Asp, Gln y Ser;

Lys sustituida por uno de Lys, Glu, Gln, His y Arg;

Asp sustituida por uno de Asp, Glu y Asn;

Glu sustituida por uno de Glu, Asp, Lys, Asn, Gln, His y Arg;

Met sustituida por uno de Met, Phe, Ile, Val, Leu y Tyr.

Una secuencia de nucleótido homóloga de conformidad con la presente invención se puede referir a secuencias de nucleótido de más de 50, 100, 200, 300, 400, 500, 600, 800 ó 1000 nucleótidos capaces de hibridizar al complemento inverso de la secuencia de nucleótido capaz de codificar la secuencia original, bajo condiciones de hibridización rigurosas (tal como las descritas por Sambrook et al., Molecular Cloning, Laboratory Manuel, Cold

Spring, Harbor Laboratory press, New York).

Como se usa en este documento, la porción funcional se refiere a una secuencia de un nanocuerpo que de suficiente tamaño de forma tal que la interacción de interés es mantenida con afinidad de 1×10^{-6} M o mayor.

Alternativamente, una porción funcional comprende una supresión parcial de la secuencia de aminoácido completa y aún mantiene los sitios enlazantes y dominios de proteína necesarios para el enlace de e interacción con el objetivo.

Como se usa en este documento, una porción funcional se refiere a menos del 100% de la secuencia completa (por ejemplo, 99%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, 1% etc.), pero comprende 5 o más aminoácidos o 15 o más nucleótidos.

Objetivos como se mencionan en este documento tal como TNF-alfa, receptor TNF-alfa, proteínas de suero (por ejemplo, albúmina de suero, inmunoglobulinas de suero, proteína que se enlaza a tiroxina, transferrina, fibrinógeno) e IFN-gama, receptor IFN-gama pueden ser fragmentos de los objetivos. Así un objetivo también es un fragmento del objetivo, capaz de provocar una respuesta inmune. Un objetivo es también un fragmento del objetivo, capaz de enlazarse a un nanocuerpo planeado contra el objetivo de longitud completa.

1056

Un fragmento como se usa en este documento, se refiere a menos del 100% de la secuencia (por ejemplo, 99%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, etc.) pero que comprende 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 o más aminoácidos. Un fragmento es de longitud suficiente de forma tal que la interacción de interés se mantiene con afinidad de 1 x 10^{-6} M o mayor.

Un fragmento como se usa en este documento, se refiere a inserciones, supresiones y sustituciones opcionales de uno o más aminoácidos las cuales no alteran sustancialmente la capacidad del objetivo a enlazarse a un nanocuerpo planeado contra el objetivo de tipo nativo. El número de inserciones, supresiones o sustituciones de aminoácido es preferiblemente hasta 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69 ó 70 aminoácidos.

Una secuencia homóloga de la presente invención puede incluir un polipéptido anti-THF-alfa el cual se ha humanizado. La humanización de anticuerpos de la nueva clase de V_{HH} podrá además reducir la posibilidad de reacción inmunología indeseada en un individuo humano de la administración.

1057

Una modalidad de la presente invención, se refiere a un método para preparar péptidos modificados en base a anticuerpos llama, reduciendo los residuos de aminoácido del dominio variable de anticuerpo (V_{HH}), el cual puede ser modificado son disminuir la afinidad nativa del dominio por antígeno y mientras se reduce su inmunogenicidad con respecto a especies heterólogas; el uso de V_{HH} que tienen modificaciones en los residuos identificados, las cuales son útiles para administración e especies heterólogas; y al V_{HH} así modificado.

Más específicamente, la invención se refiere a la preparación de V_{HH} modificados, los cuales son modificados por administración a humanos, los mismos V_{HH} resultantes, y el uso de tales V_{HH} "humanizados" en el tratamiento de enfermedades en humanos. Por humanizado significa mutado, para que en inmunogenicidad en administración a pacientes humanos es menor o no existente. Humanizando un polipéptido, de conformidad con la presente invención, comprende una etapa de reemplazar uno o más de los aminoácidos Camelidae por su contraparte humana, comprende una etapa de reemplazar uno o más de los aminoácidos Camelidae por su contraparte humana como se encuentra la secuencia consenso humana, son que el polipéptido pierda su carácter típico, es decir, la humanización no afecta significantemente la capacidad de enlace del antígeno del

polipéptido resultante. Tales métodos son bien conocidos por destinatario experto.

La humanización de nanocuerpos Camelidae requieren la introducción y mutagénesis de una cantidad limita de aminoácidos en una cadena de polipéptido única. Esto es en contraste para humanización de scFv, Fab, (Fab)2 e IgG, los cuales requieren la introducción de cambios de aminoácido en dos cadenas, la cadena ligera y la pesada y la preservación del montaje de ambas cadenas.

Como se describe en el documento WO 04/041862, se puede humanizar nanocuerpo anti-TNF. La humanización puede por ejemplo involucrar mutagénesis de residuos en FR1 en posición 1 y 5, los cuales se introducen por el cebador usado para el repertorio de clonación y no se origina de forma natural en la secuencia llama. La mutagénesis de aquellos residuos no resulta en pérdida actividad enlazante y/o inhibitoria. La humanización también puede involucrar mutagénesis de residuos en FR3 en posición 74, 76, 83, 84, 93. La mutagénesis de aquellos residuos no resulta en una pérdida dramática de actividad enlazante y/o inhibitoria. La combinación de mutaciones de FR1 y FR3 por lo tanto no afecta a la actividad enlazante o inhibitoria. La humanización también involucra mutagénesis de residuos en FR4 en posición 108. La mutagénesis de Q108L resulta en nivel de producción menor en *E. coli*. La posición 108 es

solvente expuesto en V_{HH} Camélido, mientras en anticuerpos humanos esta posición es metida en la interface VH-VL (Spinelli, 1996; Nieba, 1997). En VH aislados en posición 108 es solvente expuesto. La introducción de un Leu hidrofóbico no polar en lugar de Gln no cargado polar puede tener un efecto drástico en el plegado/estabilidad intrínseca de la molécula. También, el reemplazo de residuos hidrofílicos por residuos hidrofóbicos humanos en posiciones 44 y 45 (E44G y R45L), no tiene un efecto en enlace y/o inhibición. Sin embargo, se observó la pérdida de actividad enlazante y/o inhibición cuando se introduce F37V y F47W. Los datos de modelado confirman el residuo crítico 37 para preservar la integridad de la confirmación de bucle CDR3 y por lo tanto en actividad (toda la numeración de conformidad con el Kabat).

De acuerdo a una modalidad de la presente invención, la humanización involucra el reemplazo de cualquiera de los siguientes residuos ya sea solo o en combinación:

- FR1 en posición 1, 5, 28 y 30,
- el aminoácido marca en posición 44 y 45 en FR2,
- FR3 de residuos 74, 75, 76, 83, 84, 93 y 94,
- y en posiciones 103, 104, 108 y 111 en FR4;
- numeración de conformidad con la numeración Kabat.

1060

Una modalidad de la presente invención es un polipéptido anti-TNF-alfa, o un ácido nucleico capaz de codificar el polipéptido para uso en el tratamiento, prevención y/o alivio de los síntomas de trastornos relacionados a procesos inflamatorios. TNF-alfa se involucra en procesos inflamatorios, y el bloqueo de acción de TNF-alfa puede tener un efecto anti-inflamatorio. TNF-alfa se involucra en procesos inflamatorios, y el bloqueo de acción TNF-alfa puede tener un efecto anti-inflamatorio, el cual es muy deseable en ciertos estados de enfermedad tal como, por ejemplo, enfermedad de Crohn. Los ejemplos demuestran que V_{HH} de conformidad con la invención, los cuales enlazan a TNF-alfa y sin embargo, bloquea su enlace al receptor TNF-alfa.

Los polipéptidos anti-TNF-alfa de la presente invención se aplican a enfermedades autoinmunes, tales como enfermedad de Addison (adrenal), enfermedades autoinmunes del oído (oído), enfermedades autoinmunes del ojo (ojo), hepatitis autoinmunes (hígado), parotitis autoinmune (glándula paratiroides), enfermedad de Crohn (intestino), Diabetes tipo I (páncreas), epididimitis (epididimis), glomerulonefritis (riñón), enfermedad de Grave (tiroides), síndrome de Guillain-Barre (células nerviosas), enfermedad de Hashimoto (tiroides), anemia hemolítica (células sanguíneas rojas), lupus eritematoso sistémico (tejidos

1061

múltiples), infertilidad en machos (esperma), esclerosis múltiple (células nerviosas), Myasthenia Gravis (articulaciones neuromuscular), Pénfigos (piel principalmente), Psoriasis (piel), fiebre reumática (corazón y articulaciones), artritis reumatoide (revestimiento de articulaciones), Sarcoidosis (tejidos y órganos múltiples), Escleroderma (piel y tejidos conectivos), síndrome de Sorben (glándulas exocrinas y otros tejidos), Espondilo artropatías (esqueleto axial y otros tejidos), tiroiditis (tiroides), vasculitis (vasos sanguíneos).

Dentro de paréntesis está el tejido afectado por la enfermedad. Este listado de enfermedades autoinmunes pretende ser ejemplar más que inclusivo.

Las condiciones autoinmunes para las cuales los polipéptidos anti-TNF-alfa de la presente invención son aplicables incluyen, por ejemplo, SIDA, alergia atópica, asma bronquial, eczema, lepra, esquizofrenia, depresión heredada, transplante de tejidos y órganos, síndrome de de fatiga crónica, enfermedad de Alzheimer, enfermedad de Parkinson, infarto al miocardio, apoplejía, autismo, epilepsia, fenómeno de Arthusm anafilaxis y adicción al alcohol y drogas. En las condiciones autoinmunes definidas anteriormente, el tejido afectado es el objetivo primario, en otros casos es el objetivo secundario. Estas condiciones

1082

son síndromes parcialmente o principalmente autoinmunes. Por lo tanto, en tratarlos, es posible el uso de los mismos métodos, o aspectos de los mismos métodos y que se describe en este documento, algunas veces en combinación con otros métodos.

Otra modalidad de la presente invención es un uso de un polipéptido anti-TNF-alfa de conformidad con la invención, o un ácido nucleico capaz de codificar el polipéptido para la preparación de un medicamento para tratar un trastorno que se relaciona a procesos inflamatorios. Ejemplos de trastornos incluyen artritis reumatoide, enfermedad de Crohn, colitis ulcerativa, síndrome del intestino inflamatorio y esclerosis múltiple.

Polipéptidos y ácidos nucleicos que codifican a la presente invención pueden ser administrados a un sujeto por rutas convencionales, tal como intravenosamente, Sin embargo, una propiedad especial de los polipéptidos anti-TNF-alfa de la invención es que penetran barreras, tal como membranas de tejido y/o tumores que actúan localmente sobre este, y son suficientemente estables para resistir ambientes extremos, tal como en el estómago. Por lo tanto, otro aspecto de la presente invención que se relaciona al suministro de polipéptidos anti-TNF-alfa.

Cuando los nanocuerpos y/o polipéptidos de la invención se usan para, o se planean para uso en, la

prevención o tratamiento de enfermedades y trastornos del tracto gastrointestinal, en particular por medios de administración oral u otra administración en el tracto gastrointestinal, no será usualmente necesario usar polipéptidos de la invención que tienen vida media incrementada en suero (es decir, que se han pegilado o que contienen un Nanocuerpo dirigido contra una proteína de suero). Así, para tales indicaciones, los polipéptidos de la invención se pueden usar que únicamente contienen Nanocuerpos de la invención. En particular, se ha encontrado que para administración oral para la prevención y tratamiento de enfermedades o trastornos del tracto gastro-intestinal asociados con y/o mediados por TNF-alfa (tal como IBD y otras enfermedades y trastornos del tracto gastro-intestinal mencionado anteriormente), puede ser preferido el uso de un Nanocuerpo monovalente de la invención o de un polipéptido de la invención que esencialmente consiste de un Nanocuerpo monovalente de la invención. Para otras indicaciones, tal como tratamiento de artritis reumatoide (RA), se puede preferir el uso de un Nanocuerpo bivalente de la invención. Cuando tal Nanocuerpo alcanza su sitio planeado de acción vía la corriente sanguínea, se pude preferir el uso de un polipéptido de la invención es que incrementa su vida media en suero.

Un sujeto de conformidad con la invención puede

ser cualquier mamífero susceptible a tratamiento por polipéptidos terapéuticos.

El suministro terapéutico de polipéptidos anti-TNF-alfa de la invención, resulta en la provisión de tales moléculas en una forma activa en el colon en sitios locales que son afectados por el trastorno. Estos sitios pueden estar muy inflamados y contienen células que producen TNF-alfa. Los polipéptidos anti-TNF-alfa de la invención, los cuales se unen a TNF-alfa pueden neutralizar el TNF-alfa localmente, evitando la distribución a través del cuerpo completo y así limitando los efectos colaterales negativos. Microorganismos genéticamente modificados tal como Micrococos lactis son capaces para secretar anticuerpos o porciones funcionales del mismo. Tales microorganismos modificados se pueden usar como vehículos para producción local y suministro de anticuerpos o porciones funcionales del mismo en el intestino. Usando una cepa la cual produce un polipéptido anti-TNF-alfa, se puede tratar el síndrome del intestino inflamado.

Otro aspecto de la invención involucra polipéptidos anti-TNF suministrados, usando expresión de superficie en o secreción de bacteria no invasiva, tal como organismos hospedantes Gram positivos similares a Lactococcus spec., usando un vector tal como se describe en el documento WO02/23471.

1065

Otra modalidad de la presente invención es un polipéptido anti-TNF-alfa como se describe en este documento para uso en el tratamiento, prevención y/o alivio de síntomas de trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención, el cual es capaz de pasar a través del ambiente gástrico sin que la sustancia sea inactivada.

Ejemplos de trastornos son cualquiera que provoca inflamación, que incluyen pero no se limitan a artritis reumatoide, enfermedad de Crohn, colitis ulcerativa, síndrome del intestino inflamatorio y esclerosis múltiples. Como se conoce por persona expertas en la técnica, una vez en posesión del constructo de polipéptido, se puede aplicar tecnología de formulación para liberar una cantidad máxima de polipéptido en la ubicación derecha (en el estómago, en el colon, etc.). Este método de suministro es importante para tratar, prevenir y/o aliviar los síntomas de trastornos cuyos objetivos se localizan en el sistema del intestino.

Un aspecto de la invención es un método para tratar, prevenir y/o aliviar los síntomas de un trastorno susceptible para modulación por un Nanocuerpo o polipéptido de la invención, el cual es capaz de pasar a través del ambiente gástrico sin ser inactivado, por administración oral a un sujeto un polipéptido anti-TNF alfa como se

describe en este documento.

Otra modalidad de la presente invención es un uso de un polipéptido anti-TNF-alfa como se describe en este documento para la preparación de un medicamento para tratar, prevenir y/o aliviar los síntomas de trastornos susceptibles para modulación por un Nanocuerpo o polipéptido de la invención, el cual es capaz de pasar a través del ambiente gástrico sin ser inactivado.

Un aspecto de la invención es un método para suministrar un Nanocuerpo o polipéptido de la invención al sistema del intestino, sin que la sustancia sea inactivada, por administración oral a un sujeto un polipéptido anti-TNF-alfa como se describe en este documento.

Un aspecto de la invención es un método para suministro de un Nanocuerpo o polipéptido de la invención a la corriente sanguínea de un sujeto son que la sustancia sea inactivada, por administración oral a un sujeto un polipéptido anti-TNF-alfa como se describe en este documento para uso en el tratamiento, prevención y/o alivio de los síntomas o trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención suministrado al tracto vaginal y/o rectal.

Ejemplos de trastornos son cualquiera que causa inflamación, que incluyendo, pero no se limita a artritis reumatoide, enfermedad de Crohn, colitis ulcerativa,

síndrome del intestino inflamatorio y esclerosis múltiple. En un ejemplo no limitante, una formulación de conformidad con la invención, comprende un polipéptido anti-TNF-alfa como se describe en este documento, en la forma de un gel, crema, supositorio, película o en la forma de una esponja o un anillo vaginal que disminuye la liberación del ingrediente activo durante un tiempo (tales formulaciones se describe en los documentos EP 707473, EP 684814, US 5629001).

Un aspecto de la invención es un método para tratar, prevenir y/o aliviar los síntomas de trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención suministrado al tracto vaginal o rectal, administrando vaginalmente y/o rectalmente a un sujeto un polipéptido anti-TNF-alfa como se describe en este documento.

Otra modalidad de la presente invención es un uso de un polipéptido anti-TNF-alfa como se describe en este documento, para la preparación de un medicamento para tratar, prevenir y/o aliviar los síntomas de trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención suministrado al tracto vaginal y/o rectal.

Un aspecto de la invención es un método para suministrar un Nanocuerpo o polipéptido de la invención al tracto vaginal y/o rectal sin ser la sustancia inactivada,

administrando al tracto vaginal y/o rectal de un sujeto un polipéptido anti-TNF-alfa como se describe en este documento.

Un aspecto de la invención es un método para suministrar un Nanocuerpo o polipéptido de la invención a la corriente sanguínea de un sujeto son ser la sustancia inactivada, administrando al tracto vaginal y/o rectal de un sujeto, un polipéptido anti-TNF-alfa como se describe en este documento.

Otra modalidad de la presente invención es un polipéptido anti-TNF-alfa como se describe en este documento, para uso en el tratamiento, prevención y/o alivio de los síntomas de trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención suministrado a la nariz, tracto respiratorio superior y/o pulmón.

Ejemplos de trastornos son cualquiera que provoca inflamación, que incluye, pero no se limita a artritis reumatoide, enfermedad de Crohn, colitis ulcerativa, síndrome del intestino inflamatorio y esclerosis múltiple. En un ejemplo no limitante, una formulación de conformidad con la invención, comprende un polipéptido anti-TNF-alfa como se describe en este documento en la forma de una atomizador nasal (por ejemplo, aerosol), o inhalador. Ya que el constructo de polipéptido es pequeño, puede alcanzar

su objetivo mucho más efectivamente que las moléculas IgG terapéuticas.

Un aspecto de la invención es un método para tratar, prevenir y/o aliviar los síntomas de trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención, suministrado al tracto respiratorio superior y/o pulmón, administrando a un sujeto un polipéptido anti-TNF-alfa, como se describe en este documento, por inhalación a través de la boca o nariz.

Otra modalidad de la presente invención es un uso de un polipéptido anti-TNF-alfa como se describe en este documento para la preparación de un medicamento para tratar, prevenir y/o aliviar los síntomas de trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención suministrado a la nariz, tracto respiratorio superior y/o pulmón, sin que el polipéptido sea inactivado.

Un aspecto de la invención es un método para suministrar un Nanocuerpo o polipéptido de la invención a la nariz, tracto respiratorio superior y pulmón sin activación, administrando a la nariz, tracto respiratorio superior y/o pulmón a un sujeto un polipéptido anti-TNF-alfa como se describe en este documento.

Un aspecto de la invención es un método para suministrar un Nanocuerpo o polipéptido de la invención a

1070

la corriente sanguínea de un sujeto sin activación, administrando a la nariz, tracto respiratorio superior y/o pulmón de un sujeto un polipéptido anti-TNF-alfa como se describe en este documento.

Una modalidad de la presente invención es un polipéptido anti-TNF-alfa como se describe en este documento, para uso en el tratamiento, prevención y/o alivio de los síntomas de trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención suministrado a la mucosa intestinal, en donde el trastorno incrementa la permeabilidad de la mucosa intestinal. Porque si tamaño pequeño, un polipéptido anti-TNF-alfa como se describe en este documento, puede pasar a través de la mucosa intestinal y alcanza la corriente sanguínea más eficientemente en sujetos que sufren de trastornos los cuales causan un incremento en la permeabilidad de la mucosa intestinal, por ejemplo enfermedad de Crohn.

Un aspecto de la invención es un método para tratar, prevenir y/o aliviar los síntomas de trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención suministrado a la mucosa intestinal, en donde tal trastorno incrementa la permeabilidad de la mucosa intestinal, administración oral a un sujeto un polipéptido anti-TNF-alfa como se describe en este documento.

1071

Este proceso puede aún ser mejorado por un aspecto adicional de la presente invención, el uso de portadores de transporte activo. En este aspecto de la invención, V_{HH} es fusionado a un portador que mejora la transferencia a través de la pared intestinal en la corriente sanguínea. En un ejemplo no limitante, este "portador" es un segundo V_{HH} el cual se fusiona al V_{HH} terapéutico. Tal constructo de fusión de hace usando métodos conocidos en la técnica. El "portador" V_{HH} se une específicamente a un receptor en la pared intestinal el cual conduce una transferencia activa a través de la pared.

Otra modalidad de la presente invención es un uso de un polipéptido anti-TNF-alfa como se describe en este documento para la preparación de un medicamento para tratar, prevenir y/o aliviar los síntomas de trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención suministrado a la mucosa intestinal, en donde el trastorno incrementa la permeabilidad de la mucosa intestinal.

Un aspecto de la invención es un método para suministrar un Nanocuerpo o polipéptido de la invención a la mucosa intestinal, sin ser inactivado, administrando oralmente a un sujeto un polipéptido anti-TNF-alfa de la invención.

Un aspecto de la invención es un método para

suministrar un Nanocuerpo o polipéptido de la invención a la corriente sanguínea de un sujeto son ser inactivada, administrando oralmente a un sujeto un polipéptido anti-TNF-alfa de la invención.

Este proceso puede ser aún además mejorado por un aspecto adicional de la presente invención, el uso de portadores de transporte activo. En este aspecto de la invención, un polipéptido anti-TNF-alfa como se describe en este documento es fusionado a un portador que mejora la transferencia a través de la pared intestinal en la corriente sanguínea. En un ejemplo no limitante, este "portador" es un V_{HH} el cual se fusiona con el polipéptido. Tales constructos de fusión se hacen usando métodos conocidos en la técnica. El "portador" V_{HH} se enlaza específicamente a un receptor en la pared intestinal, la cual induce una transferencia activa a través de la pared.

Una modalidad de la presente invención es un polipéptido anti-TNF-alfa como se describe en este documento para uso en el tratamiento, prevención y/o alivio de los síntomas de trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención, el cual es capaz de pasar a través de los tejidos abajo de la lengua efectivamente.

Ejemplos de trastornos son cualquiera que causan inflamación, que incluyen, pero no se limitan a artritis

reumatoide, enfermedad de Crohn, colitis ulcerativa, síndrome del intestino inflamatorio y esclerosis múltiple. Una formulación del constructo del péptido como se describe en este documento, por ejemplo, una tableta, un atomizador, una gota se coloca bajo la lengua y se adsorbe a través de las membranas de mucosa en la red capilar bajo la lengua.

Un aspecto de la invención es un método para tratar, prevenir y/o aliviar los síntomas de trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención, el cual es capaz de pasar a través de tejidos abajo de la lengua efectivamente, por administración sublingualmente a un sujeto un polipéptido anti-TNF-alfa como se describe en este documento.

Otra modalidad de la presente invención es un uso de un polipéptido anti-TNF-alfa como se describe para la preparación de un medicamento para tratar, prevenir y/o aliviar los síntomas de trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención, el cual es capaz de pasar a través de los tejidos abajo de la lengua.

Un aspecto de la invención es un método para suministro de un Nanocuerpo o polipéptido de la invención a los tejidos debajo de la lengua son ser inactivado, administrando sublingualmente a un sujeto un polipéptido anti-TNF-alfa como se describe en este documento.

Un aspecto de la invención es un método para suministrar un Nanocuerpo o polipéptido de la invención a la corriente sanguínea de un sujeto son ser inactivado, administrando oralmente a un sujeto un polipéptido anti-TNF-alfa como se describe en este documento.

Una modalidad de la presente invención es un polipéptido anti-TNF-alfa como se describe en este documento para uso en el tratamiento, prevención y/o alivio de los síntomas de trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención, el cual es capaz de pasar a través de la piel efectivamente.

Ejemplos de trastornos son cualquiera que causan inflamación, que incluyen, pero no se limitan a artritis reumatoide, enfermedad de Crohn, colitis ulcerativa, síndrome del intestino inflamado y esclerosis múltiple. Una formulación del constructo de polipéptido, por ejemplo, una crema, película, atomizador, gota, parche, se coloca en la piel y pasa a través de esta.

Un aspecto de la invención es un método para tratar, prevenir y/o aliviar los síntomas de trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención, el cual es capaz de pasar a través de la piel efectivamente, administrando tópicamente a un sujeto un polipéptido anti-TNF-alfa como se describe en este documento.

1075

Otra modalidad de la presente invención es un uso de un polipéptido anti-TNF-alfa como se describe en este documento para la preparación de un medicamento para tratar, prevenir y/o aliviar los síntomas de trastornos susceptibles a modulación por un Nanocuerpo o polipéptido de la invención el cual es capaz de pasar a través de la piel efectivamente.

Un aspecto de la invención es un método para suministrar un Nanocuerpo o polipéptido de la invención a la piel sin ser inactivado, administrando tópicamente a un sujeto un polipéptido anti-TNF-alfa como se describe en este documento.

Un aspecto de la invención es un método para suministrar un Nanocuerpo o polipéptido de la invención a la corriente sanguínea de un sujeto, administrando tópicamente a un sujeto un polipéptido anti-TNF-alfa como se describe en este documento.

En otra modalidad de la presente invención, un polipéptido anti-TNF-alfa además comprende un nanocuerpo portador (por ejemplo, V_{HH}), el cual actúa como un portador de transporte activo para transportar el polipéptido anti-TNF-alfa, a partir del lumen de pulmón a la sangre.

Un polipéptido anti-TNF-alfa además comprende un portador enlazado específicamente a un receptor presente en la superficie de la mucosa (células epiteliales

1076

bronquiales) que resulta en el transporte activo del polipéptido del lumen del pulmón a la sangre. El nanocuerpo portador puede ser fusionado al constructo del polipéptido. Tales constructos de fusión se pueden hacer usando métodos conocidos en la técnica y se describen en este documento. El nanocuerpo "portador" se enlaza específicamente a un receptor en la superficie de la mucosa, la cual induce una transferencia activa a través de la superficie.

Otro aspecto de la presente invención es un método para determinar los anticuerpos (por ejemplo, V_{HH}) son activamente transportado en la corriente sanguínea en administración nasal. Similarmente, una biblioteca del fago V_{HH} nativo o inmune se puede administrar nasalmente, y después de puntos de tiempo diferente después de la administración, sangre y órganos se pueden aislar para rescatar fagos que son activamente transportados en la corriente sanguínea. Un ejemplo no limitante de un receptor para transporte activo del lumen de pulmón a la corriente sanguínea es el receptor Fc (FcRn). Un aspecto de la invención incluye las moléculas V_{HH} identificadas por el método. Tal V_{HH} puede después ser usado como un portador V_{HH} para el suministro de un V_{HH} terapéutico al objetivo correspondiente en la corriente sanguínea en administración nasal.

En un aspecto de la invención, uno puede usar un

polipéptido anti-TNF-alfa como se describe en este documento, para modular los agentes que modula el enlace del péptido a TNF-alfa. Cuando se identifica en un ensayo que mide el enlace o solo el desplazamiento solo, agentes que pueden ser sometidos a pruebas funcionales para determinar sin modulan la acción del antígeno in vivo.

En un ejemplo de un experimento de desplazamiento, fago o células que expresan TNF-alfa o un fragmento del mismo, se inoculan en amortiguador enlazante con polipéptido de la invención, el cual se ha etiquetado, en la presencia o ausencia de concentraciones incrementadas de un modulador candidato. Para validar y calibrar el ensayo, se pueden realizar reacciones de competición control usando concentraciones incrementadas del polipéptido y el cual no es etiquetado. Después de la incubación, las células se lavan extensivamente, y se unen, el polipéptido etiquetado se mide apropiadamente para la etiqueta proporcionada (por ejemplo, conteo de escintilación, fluorescencia, etc.). Una disminución de al menos 10% en la cantidad del polipéptido etiquetado enlazado en la presencia del modular candidato, indica desplazamiento de enlace por el modular candidato. Los moduladores candidatos se consideran enlazar específicamente en este u otros ensayos descritos en este documento si se desplazan al 50% de polipéptido etiquetado

1077

(dosis del polipéptido sub-saturado) a una concentración de 1 μM o menos.

Alternativamente, el enlace o desplazamiento del enlace se puede monitorear por superficie de plasmón de superficie (SPR). Se pueden usar ensayos de resonancia de plasmón de superficie como un método cuantitativo para enlazarse entre dos moléculas por el cambio en masa casi un sensor inmovilizado causado por el enlace o pérdida de enlace del polipéptido de la invención de la fase acuosa para TNF-alfa inmovilizado en una membrana en el sensor. Este cambio de masa se mide como unidades de resonancia contra tiempo, después de la inyección o remoción del polipéptido o modulador candidato y se mide usando un Biosensor Biacore (Biacore AB). TNF-alfa puede ser por ejemplo inmovilizado en un chip sensor (por ejemplo, chip CM5 grado de investigación; Biacore AB) en una membrana de lípido de película delgada de conformidad con métodos descritos por Salamon et al., (Salamon et al., 1996, Biophys J. 71: 283-294; Salamon et al., 2001, Biophys. J. 80: 1557-1567; Salamon et al., 1999, Trends Biochem. Sci. 24: 213-219, cada uno de los cuales se incorpora en este documento por referencia). Sarrio et al., demuestra que se puede usar SPR para detectar ligandos enlazado el receptor de adenosina GPCR A(1) inmovilizado en una capa de lípido en el chip (Sarrio et al., 2001, Mol. Cell. Biol. 20: 5164-

1079

5174, incorporado en este documento por referencia). Las condiciones para en enlace de un polipéptido de la invención a TNF-alfa en un ensayo SPR puede ser sintonizado finamente por un experto en la técnica usando las condiciones reportadas por Sarrio et al., como un punto de partida.

El SPR se puede someter a ensayo por moduladores de enlace en al menos dos vías. Primero, un polipéptido de la invención puede ser pre-enlazado para inmovilizar TNF-alfa, seguido por inyección del modulador candidato a una concentración que varía de 0.1 nM a 1 µM. El desplazamiento del polipéptido enlazado se puede cuantificar, permitiendo detecciones del enlace del modulador. Alternativamente, el TNF-alfa enlazado a la membrana puede ser pre-incubada con un modulador candidato y cambiado en el polipéptido de la invención. Una diferencia en afinidad de enlace entre el polipéptido y TNF-alfa pre-incubado con el modulador, comparado entre el polipéptido y TNF-alfa en ausencia del modulador demostrará enlace o desplazamiento del polipéptido en la presencia del modulador. En cualquier ensayo, una disminución del 10% o más en la cantidad del enlace del polipéptido en la presencia del modulador candidato, en relación a la cantidad del enlace de polipéptido en la ausencia del modulador candidato indica que el modulador candidato inhibe la interacción de TNF-

alfa y el polipéptido.

Otro método para detectar la inhibición del enlace de, por ejemplo, un polipéptido de la invención, para TNF-alfa que usa transferencia de energía de resonancia de fluorescencia (FRET). FRET es un fenómeno mecánico quantum que ocurre entre un donador de fluorescencia (D) y un aceptor fluorescente (A) en proximidad cerrada entre sí (usualmente < 100 Å de separación) si el espectro de emisión de traslapes D con el espectro de excitación de A. Las moléculas a ser probadas, por ejemplo, un polipéptido de la invención y un TNF-alfa se etiquetan con un par complementario del donador y fluoróforos aceptables. Mientras cercanamente enlazado junto con el TNF-alfa: polipéptido de interacción, la fluorescencia emitida en excitación del donador de fluoróforo, podrá tener una longitud de onda diferente de la emitida en respuesta a que la longitud de onda de excitación cuando el polipéptido y TNF-alfa no se enlazan, proporcionado para cuantificación de moléculas enlazadas contra no enlazadas por medición de intensidad de emisión en cada longitud de onda. Los fluoróforos donadores con los cuales se etiqueta el TNF-alfa son bien conocidos en la técnica. De interés particular son variantes de A. Se conoce GFP victoria como Cian FP (CFP, Donador (D)) y Amarillo FP (YFP, Aceptor (A)). Como un ejemplo, la

variante YFP se puede hacer como una proteína de fusión con TNF-alfa. Vectores para la expresión de variantes GFP como fusiones (Clontech), así como reactivos etiquetados con fluoróforo (Molecular Probes) se conoce en la técnica. La adición de un modulador candidato a la mezcla de polipéptido etiquetado fluorescentemente y YFP-TNF-alfa resultará en una inhibición de evidencia de transferencia de energía, por ejemplo, una enfermedad en fluorescencia en relación a una muestra sin el modulador candidato. En un ensayo usando FRET para la detección de interacción TNF-alfa:polipéptido, un 10% o mayor disminución en la intensidad de emisión fluorescente en la longitud de onda aceptora en muestras que contienen un modulador candidato, en relación a muestras sin el modulador candidato, indica que el modulador candidato inhibe la interacción de TNF-alfa:polipéptido.

Una muestra como se usa en este documento puede ser una muestra biológica que contiene TNF-alfa tal como clínico (por ejemplo, fracciones celulares, sangre completa, plasma, suero, tejido, células, etc.), derivado de muestras clínicas, agricultura, forense, investigación u otra posible. Las muestras clínicas pueden ser de origen humano o animal. La muestra analizada puede ser tanto sólida como líquida en naturaleza. Es evidente cuando se usan materiales sólidos, estos son primero disueltos en una

solución adecuada.

Una variación en FRET usa apagado fluorescente para interacciones moleculares menores. Una molécula en el par que interactúa puede ser etiquetado con un fluoróforo, y el otro una molécula que apaga la fluorescencia del fluoróforo cuando en aposición cerrada con el. Un cambio en fluorescencia en excitación es indicativo de un cambio en la asociación de las moléculas etiquetadas con el par fluoróforo:apagador. Generalmente, un incremento en fluorescencia del TNF-alfa etiquetado es indicativo que el polipéptido anti-TNF-alfa que porta el apagador se ha desplazado. Para ensayos de apagado, un 10% o mayor incremento en la intensidad de emisión fluorescente en muestras que contienen un modulador candidato, en relación a muestras son el modulador candidato, indica que el modulador candidato inhibe la interacción de polipéptido TNF-alfa:anti-TNF-alfa.

Además a la resonancia de plasmon de superficie y métodos FRET, se utiliza la medición de polarización fluorescente para cuantificar en enlace. El valor de polarización fluorescente para una molécula etiquetada fluorescentemente depende en el tiempo de correlación rotacional o velocidad movimiento de rotación. Complejos, tal como aquellos formados por TNF-alfa en asociación con un polipéptido anti-TNF-alfa fluorescentemente etiquetado,

tiene valores de polarización mayor que el polipéptido etiquetado, no sometido a complejo. La inclusión de un inhibidor candidato de la interacción de polipéptido THF-alfa:anti-TNF-alfa resulta en una disminución en polarización fluorescente, en relación a una mezcla sin el inhibidor candidato, si el inhibidor candidato afecta o inhibe la interacción de TNF-alfa con el polipéptido. La polarización fluorescente es bien adecuada para identificación de moléculas pequeñas que afectan la formación de complejos de polipéptido TNF-alfa:anti-TNF-alfa. Una disminución del 10% o más en polarización fluorescente en muestras que contienen un modulador candidato, en relación a polarización fluorescente en una muestra que carece del modulador candidato, indica que el modulador candidato inhibe la interacción de polipéptido TNF-alfa:anti-TNF-alfa.

Otra alternativa para monitorear interacciones de polipéptido TNF-alfa:anti-TNF-alfa usa un ensayo biosensor. Se han descrito biosensores ICS en la técnica (Australian Membrane Biotechnology Research Institute; Cornell B, Braach-Maksvytis V, King L, Osman P, Raguse B, Wieczorek L, y Pace R. "A biosensor that uses ion-channel switches" Nature 1997, 387, 580). En esta tecnología, la asociación de TNF-alfa y un polipéptido anti-TNF-alfa se acopla al cierre de canales de ión facilitado de gramacidina en

bicapas de membrana suspendida y así a un cambio medible en la admisión (similar a impedancia) del biosensor. Esta propuesta es lineal sobre seis ordenes de magnitud de cambio de admisión y es idealmente adecuada para escala mayor, selección de mayor procedimiento de bibliotecas de combinación de molécula pequeña. Un cambio del 10% o mayor (incremento o disminución) en admisión en una muestra que contiene un modulador candidato, en relación a la admisión de una muestra que carece el modulador candidato, indica que el modulador candidato inhibe la interacción de TNF-alfa y el polipéptido. Es importante notar que en ensayos para probar la interacción de TNF-alfa con un polipéptido anti-TNF-alfa, es posible que un modulador de interacción no necesita necesariamente interactuar directamente con los dominios de las proteínas que interactúan físicamente con el polipéptido. También es posible que un modulador podrá interactuar en una ubicación removida del sitio de interacción y causa, por ejemplo, un cambio conformacional en el TNF-alfa. Moduladores (inhibidores o agonistas) que actúan en esta manera son no obstante de interés como agentes para modular el enlace de TNF-alfa a su receptor.

Cualquiera de los ensayos de enlace descritos se pueden usar para determinar la presencia de un agente en una muestra, por ejemplo, una muestra de tejido, que se enlaza a TNF-alfa, o que afecta el enlace de, por ejemplo,

un polipéptido de la invención al TNF-alfa. Se hace reaccionar un TNF-alfa con el polipéptido en la presencia o ausencia de la muestra, y se mide el enlace del polipéptido como apropiado para el ensayo de enlace siendo usado. Una disminución del 10% o más en el enlace del polipéptido indica que la muestra contiene un agente que modula en enlace del polipéptido al TNF-alfa. Por supuesto, el método anteriormente generalizado puede fácilmente aplicado para seleccionar los moduladores candidatos los cuales alteran el enlace entre cualquier polipéptido anti-TNF-alfa de la invención, una secuencia homóloga del mismo, una porción funcional del mismo o una porción funcional de una secuencia homóloga del mismo, y TNF-alfa o un fragmento del mismo.

Una modalidad de la presente invención es un agente desconocido identificado por el método descrito en este documento.

Una modalidad de la presente invención es un agente desconocido identificado por el método descrito en este documento para uso en el tratamiento, prevención y/o alivio de los síntomas de trastornos relacionados de procesos inflamatorios.

Otra modalidad de la presente invención es un uso de un agente desconocido identificado por el método descrito en este documento para uso en el tratamiento,

prevención y/o alivio de los síntomas de trastornos relacionados a un proceso inflamatorio.

Ejemplos de trastornos incluyen artritis reumatoide, enfermedad de Crohn, colitis ulcerativa, síndrome del intestino inflamatorio y esclerosis múltiple.

Una célula que es útil de conformidad con la invención es preferiblemente seleccionada del grupo que consiste de células bacterianas tal como, por ejemplo, *E. coli*, células de levadura tal como, por ejemplo, S. cerevisae, P. pastoris, células de insecto o células de mamífero.

Una célula que es útil de conformidad con la invención puede ser cualquier célula en la cual una secuencia de ácido nucleico que codifica un polipéptido comprende un TNF-alfa de la invención, una secuencia homóloga del mismo, una porción funcional del mismo o una porción funcional de una secuencia homóloga del mismo, de conformidad con la invención se puede introducir de forma tal que el polipéptido se expresa en niveles naturales o abajo de niveles naturales, como se define en este documento. Preferiblemente, un polipéptido de la invención que se expresa en una célula que exhibe farmacología normal o casi normal, como se define en este documento.

De acuerdo a una modalidad preferida de la presente invención, una célula se selecciona del grupo que

consiste de células COS7, una célula CHO, una célula LM (TK-), una célula NIH-3T3, célula HEK-293, célula K-562 o una célula astrocitoma 1321N1 pero también otras líneas celulares transfectables.

En general, "cantidad terapéuticamente efectiva", "dosis terapéuticamente efectiva" y "cantidad efectiva" significa la cantidad necesaria para lograr el resultado o resultados deseados (modulación del enlace TNF-alfa; tratamiento o prevención de inflamación). Un experto de experiencia ordinaria en la técnica, reconocerá que la potencia y, por lo tanto, una "cantidad efectiva" puede variar por los compuestos variados que modulan en enlace TNF-alfa usando en la invención. Un experto en la técnica puede fácilmente valorar la potencia del compuesto.

Como se usa en este documento, el término "compuesto" se refiere a un polipéptido anti-TNF-alfa de la presente invención, una composición, o un ácido nucleico capaz de codificar el polipéptido o un agente identificado de conformidad con el método seleccionado descrito en este documento o el polipéptido comprende uno o más aminoácidos derivatizados.

Por "farmacéuticamente aceptable" significa un material que no es biológicamente o de otra forma indeseable, es decir, el material puede ser administrado a un individuo junto con el compuesto sin causar cualquier

1089

efecto biológico indeseable o que interactúa en una manera nociva con cualquiera de los otros componentes de la composición farmacéutica en la cual se contiene.

Polipéptidos anti-TNF-alfa como se describen en este documento son útiles para tratar o prevenir condiciones en un sujeto y comprende administrar una cantidad farmacéuticamente efectiva de un compuesto o composición.

Polipéptidos anti-TNF de la presente invención son útiles para tratar o prevenir condiciones que se relacionan a artritis reumatoide, enfermedad de Crohn, colitis ulcerativa, síndrome del intestino inflamatorio y esclerosis múltiple en un sujeto y comprende administrar una cantidad farmacéuticamente efectiva de un compuesto o composición que enlaza TNF-alfa.

Polipéptidos anti-TNF alfa como se describe en este documento, son útiles para tratar o prevenir condiciones en un sujeto y comprende administrar una cantidad farmacéuticamente efectiva de una combinación de compuesto con otra, tal como, por ejemplo, aspirina.

Los polipéptidos anti-TNF-alfa como se describe en este documento, son útiles para tratar o prevenir condiciones que se relacionan a artritis reumatoide, enfermedad de Crohn, colitis ulcerativa, síndrome del intestino inflamatoria y esclerosis múltiple en un sujeto y

1090

comprende administrar una cantidad farmacéuticamente efectiva de una combinación de compuesto con otra, tal como, por ejemplo, aspirina.

La presente invención no se limita a la administración de formulaciones que comprenden un compuesto único de la invención. Es dentro del alcance de la invención para proporcionar combinación de tratamientos en donde una formulación es administrada a un paciente en necesidad del mismo que comprende más de un compuesto de la invención.

Condiciones mediadas por TNF-alfa incluyen, pero no se limitan a artritis reumatoide, enfermedad de Crohn, colitis ulcerativa, síndrome del intestino inflamatorio y esclerosis múltiple.

Un compuesto útil en la presente invención puede ser formulado como composiciones farmacéuticas y administrado a un hospedante de mamífero, tal como un paciente humano o un animal doméstico en una variedad de formas adaptadas a la ruta escogida de administración, es decir, oralmente o parenteralmente, intranasalmente por inhalación, intravenosa, intramuscular, tópica o rutas subcutáneas.

Un compuesto de la presente invención puede también ser administrado usando métodos de terapia del gen de suministro. Véase, por ejemplo, Patente Estadounidense

1091

No. 5,399,346, la cual se incorpora por referencia en su totalidad. Usando un método de terapia del gen de suministro, células primarias transfectadas con el gen para el compuesto de la presente invención pueden adicionalmente ser transfectadas con promotores específicos del tejido a órganos, tejido, injerto, tumores o células específicas objetivo.

Así, el compuesto presente puede ser administrado sistémicamente, por ejemplo, oralmente, en combinación con un vehículo farmacéuticamente aceptable tal como un diluyente inerte o un portador comestible asimilable. Pueden ser adjuntados en cápsulas de gelatina dura o suave, pueden ser comprimidos en tabletas, o pueden ser incorporados directamente con el alimento de la dieta del paciente. Para administración terapéutica oral, el compuesto activo puede ser combinado con uno o más excipientes y se usan en la forma de tabletas ingeribles, tabletas bucales, trociscos, cápsulas, elixires, suspensiones, jarabes, obleas y similares. Tales composiciones y preparaciones deben contener al menos 0.1% del compuesto activo. El porcentaje de las composiciones y preparaciones puede, por supuesto, ser variado y puede convenientemente ser entre aproximadamente 2 hasta aproximadamente 60% del peso de una forma de dosificación unitaria proporcionada. La cantidad del compuesto activo en

1092

tal composición terapéuticamente útil es de forma tal que se obtendrá el nivel de dosificación efectivo.

Las tabletas, trociscos, píldoras, cápsulas y similares también pueden contener los siguiente: también se pueden agregar aglutinantes tales como goma de tragacanto, acacia, almidón de maíz o gelatina; excipientes tales como fosfato dicalcio; un agente desintegrante tal como almidón de maíz, almidón de para, ácido algínico y similares; un lubricante tal como estearato de magnesio; y un agente endulzantes tal como sacarosa, fructuosa, lactosa aspartame o un agente saborizante tal como menta, aceite de gaulteria, o saborizante de cereza. Cuando la forma de unidad de dosificación es una cápsula, puede contener además al material del tipo anterior, un portador líquido, tal como un aceite vegetal o un polietilen glicol. Otros materiales variados pueden estar presentes como revestimientos o para de otra forma modificar la forma física de la forma de dosificación unitaria sólida. Por ejemplo, tabletas, píldoras o cápsulas pueden ser revestidas con gelatina, cera, laca o azúcar y similares. Un jarabe o elixir puede contener el compuesto activo, sacarosa o fructuosa como un agente edulcorante, metilo y propilparabenos tal como preservativos, un tinte y saborizante tal como sabor cereza o naranja. Por supuesto, cualquier material usado en la preparación de cualquier

1093

forma de dosificación unitaria debe ser farmacéuticamente aceptable y sustancialmente no tóxico en las cantidades empleadas. Además, el compuesto activo puede ser incorporado en preparaciones y dispositivos de liberación sostenida.

El compuesto activo también se administra intravenosamente o intraperitonealmente por infusión o inyección. Las soluciones del compuesto activo o sus sales se pueden preparar en agua, opcionalmente mezclado con un tensoactivo no tóxico. También se pueden preparar dispersiones en glicerol, polietilen glicoles líquidos, triacetina y mezclas de los mismos y en aceites. Bajo condiciones ordinarias de almacenaje y uso, estas preparaciones contienen un preservativo para prevenir el crecimiento de microorganismos.

Las formas de dosificación farmacéutica adecuadas para inyección o infusión pueden incluir soluciones o dispersiones acuosas estériles o polvos estériles que comprenden el ingrediente activo los cuales se adaptan para la preparación extemporánea de soluciones o dispersiones inyectables o infusibles estériles, opcionalmente encapsulados en liposomas. En todos los casos, la forma de dosificación última debe ser estéril, fluida y estable bajo las condiciones de manufactura y almacenaje. El portador líquido o vehículo puede ser un solvente o medio de

1094

dispersión líquido que comprende, por ejemplo, agua, etanol, un poliol (por ejemplo, glicerol, propilen glicol, polietilen glicoles líquidos y similares), aceites vegetales, gliceril ésteres no tóxicos y mezclas adecuadas de los mismos. Se puede mantener la fluidez apropiada, por ejemplo, por la formación de liposomas, por el mantenimiento de tamaño de partículas requeridas en el caso de dispersiones o por el uso de tensoactivos. La prevención de la acción de microorganismos puede ser causado aproximadamente por varios agentes antibacterianos y antifúngicos, por ejemplo, parabenos, clorobutanol, fenol, ácido sórbico, timerosal y similares. En muchos casos, será preferible incluir agentes isotónicos, por ejemplo, azucares, amortiguadores o cloruro de sodio. Se puede provocar la absorción prolongada de las composiciones inyectables aproximadamente por el uso en las composiciones de agentes que retardan la absorción, por ejemplo, monoestearato de aluminio y gelatina.

Se preparan soluciones inyectables estériles incorporando el compuesto activo en la cantidad requerida en el solvente apropiado con varios de los otros ingredientes enumerados anteriormente, como sea requeridos, seguido por esterilización por filtrado. En el caso de polvos estériles para la preparación de soluciones inyectables estériles, los métodos preferidos de

preparación son secado a vacío y las técnicas de secado por congelamiento, las cuales proporcionan un polvo del ingrediente activo más cualquier ingrediente adicional deseado presente en las soluciones filtradas previamente esterilizadas.

Para administración tópica, el compuesto presente puede ser aplicado en forma pura, es decir, cuando son líquidos. Sin embargo, será generalmente deseable administrarlos a la piel como composiciones o formulaciones, en combinación con un portador dermatológicamente aceptable, el cual puede ser un sólido o un líquido.

Portadores sólidos útiles incluyen sólidos finamente divididos tal como talco, arcilla, celulosa microcristalina, sílice, alúmina y similares. Portadores líquidos útiles incluyen agua, hidroxialquilos o glicoles o mezclas agua-alcohol/glicol, en las cuales el compuesto presente se puede disolver o dispersar en niveles efectivos, opcionalmente con la ayuda de tensoactivos no tóxicos. Se pueden agregar adyuvantes tales como fragancias y agentes antimicrobianos adicionales para optimizar las propiedades para un uso proporcionado. Las composiciones líquidas resultantes se pueden aplicar a partir de almohadillas absorbentes, usadas para impregnar vendajes y otras telas, o rociando en el área afectada usando

rociadores tipo bomba o en aerosol.

Espesantes tales como polímeros sintéticos, ácidos grasos, sales de ácido graso y ésteres, alcoholes grasos, celulosas modificadas o materiales de mineral modificados también se pueden emplear con portadores líquidos para formar pastas para untar, geles, ungüentos, jabones y similares, para aplicación directamente a la piel del usuario.

Ejemplos de composiciones dermatológicas útiles las cuales se pueden usar para suministrar el compuesto a la piel se conoce en la técnica; por ejemplo, véase Jacquet et al., (Patente Estadounidense No. 4,608,392), Geria (Patente Estadounidense No. 4,992,478), Smith et al. (Patente Estadounidense No. 4,559,157) y Wortzman (Patente Estadounidense No. 4,820,508).

Dosificaciones útiles del compuesto se pueden determinar comparando su actividad in vitro, y actividad in vivo en modelos animales. Métodos para la extrapolación de dosificaciones efectivas en ratones y otros animales, a humanos se conoce en la técnica; por ejemplo, véase la Patente Estadounidense No. 4,938,949.

Generalmente, la concentración de los compuestos en una composición líquida, tal como una loción, será de aproximadamente 0.1-25% en peso, preferiblemente de aproximadamente 0.5-10% en peso. La concentración en una

composición semi-sólida o sólida tal como un gel o un polvo será aproximadamente 0.1-5% en peso, preferiblemente aproximadamente 0.5-2.5% en peso.

La cantidad del compuesto, o una sal activa o derivado del mismo, requerida para uso en tratamiento, variará no únicamente con la sal particular seleccionada pero también con la ruta de administración, la naturaleza de la condición a ser tratada y la edad y condición del paciente y será en última instancia en la discreción del médico o especialista que atiende. También la dosificación del compuesto varia dependiendo de la célula objetivo, tumor, tejido, injerto u órgano.

La dosis deseada puede convenientemente ser presentada en una dosis única o como dosis dividida administrada en intervalos apropiados, por ejemplo, como dos, tres, cuatro o más sub-dosis por día. La sub-dosis puede además ser dividida, por ejemplo, en un número de administraciones especiadas con discreta holgura; tal como inhalaciones múltiples de un soplador o por aplicación de una pluralidad de gotas en el ojo.

Un régimen de administración puede incluir tratamiento diario de acción prolongada. Por "acción prolongada" significa al menos dos semanas y preferiblemente, varias semanas, meses o años de duración. Se pueden determinar modificaciones necesarias en esta

intervalo de dosificación por un experto ordinario en la técnica, usando únicamente experimentación de rutina proporcionada en las enseñanzas en este documento. Véase Remington's Pharmaceutical Sciences (Martin, E.W., ed. 4), Mack Publishing Co., Easton, PA. La dosificación también se puede ajustar por el médico individual en el evento de cualquier complicación.

La invención proporcionada para un agente que es un modulador de interacciones del receptor TNF-alfa/TNF-alfa.

El agente candidato puede ser un agente sintético, o una mezcla de agentes, o puede ser un producto natural (por ejemplo, un extracto de planta o cultivo sobrenadante). Un agente candidato de conformidad con la invención incluye una molécula pequeña que puede ser sintetizada, un extracto natural, péptidos, proteínas, carbohidratos, lípidos, etc.

Se pueden seleccionar agentes moduladores candidatos de bibliotecas grandes de agentes sintéticos o naturales. Numerosos medios son actualmente usados para síntesis directa y al azar de agentes en base a sacárido, péptido y ácido nucleico. Bibliotecas de agente sintético están comercialmente disponibles de un número de compañías que incluyen Maybridge Chemical Co. (Trevillet, Cornwall, UK), Comgenex (Princeton, NJ), Brandon Associates

(Merrimack, NH) y Microsource (New Milford, CT). Una rara biblioteca química está disponible de Aldrich (Milwauke, WI). Bibliotecas combinatorias están disponibles y se pueden preparar. Alternativamente, las bibliotecas de agentes naturales en la forma de extractos de bacteria, hongo, planta y animal están disponibles de por ejemplo, Pan Laboratories (Bothell, WA) o MycoSearch (NC), o son fácilmente producibles por métodos bien conocidos en la técnica. Adicionalmente, bibliotecas y agentes producidos natural y sintéticamente son fácilmente modificados a través de medios químicos, físicos y bioquímicos convencionales.

Agentes útiles se pueden encontrar dentro de numerosas clases químicas. Agentes útiles pueden ser agentes orgánicos o agentes orgánicos pequeños. Agentes orgánicos pequeños tienen un peso molecular de más de 50 aún más de aproximadamente 2,500 daltons, preferiblemente menos de aproximadamente 750, más preferiblemente menos de aproximadamente 350 daltons. Clases ejemplares incluyen heterocitos, péptidos, sacáridos, esteroides y similares. Los agentes se pueden modificar para mejorar la eficacia, estabilidad, compatibilidad farmacéutica y similares. Se puede usar la identificación estructural de un agente para identificar, generar o seleccionar agentes adicionales. Por ejemplo, en donde los agentes del péptido se identifican,

pueden ser modificados en una variedad de vías para mejorar su estabilidad, tal como usando un aminoácido no natural, tal como aminoácido D, particularmente alanina D, funcionalizando el amino o términos carboxílicos, por ejemplo, para el grupo amino, acilación o alquilación y para el grupo carboxilo, esterificación o amidificación o similares.

Para selección primaria, una concentración útiles de un agente candidato de conformidad con la invención se de aproximadamente 10 mM hasta aproximadamente 100 µM o más (es decir, 1 mM, 10 mM, 100 mM, 1 M, etc.). La concentración de selección primaria será usada como un límite superior, a lo largo con nueve concentraciones adicionales, en donde las concentraciones adicionales se determinan reduciendo la concentración de selección primaria en intervalos diarios medios (por ejemplo, por 9 concentraciones más) para selecciones secundarias o para generar curvas de concentración.

Un kit se selección para procesamiento mayor de conformidad con la invención, comprende todos los medios necesarios y medios para realizar la detección de un agente que modula interacciones del receptor TNF-alfa/TNF-alfa, interactuando con TNF-alfa en la presencia de un polipéptido, preferiblemente a una concentración en el intervalo de 1 µM a 1 mM.

1101

El kit comprende lo siguiente: Células recombinantes de la invención, que comprenden y expresan la secuencia de nucleótido que codifica TNF-alfa, el cual se hace crecer de conformidad con el kit en un soporte sólido, tal como una placa de microtitulador, más preferiblemente una placa de microtitulador de 96 cavidades, de conformidad a métodos bien conocidos en la persona experta en la técnica, especialmente como se describe en el documento WO 00/02045. Alternativamente TNF-alfa se proporciona en una forma purificada para ser inmovilizado en, por ejemplo, una placa de microtitulador de 96 cavidades por la persona experta en la técnica. Alternativamente TNF-alfa se proporciona en el kit pre-inmovilizado en, por ejemplo, una placa de microtitulador de 96 cavidades. El TNF-alfa puede ser TNF-alfa completo o un fragmento del mismo.

Agentes moduladores de conformidad con la invención, en concentraciones de aproximadamente 1 µM hasta 1 mM o más, se agregan a las paredes definidas en la presencia de una concentración apropiada de polipéptido anti-TNF-alfa, una secuencia homóloga del mismo, una porción funcional del mismo o una porción funcional de una secuencia homóloga del mismo, dicha concentración del polipéptido preferiblemente en el intervalo de 1 µM hasta 1 mM. Kits que contienen uno o más polipéptidos anti-TNF-alfa de la invención.

1102

Ensayos de enlace se realizan de conformidad con los métodos ya descritos en este documento y los resultados se comparan al nivel de línea base de, por ejemplo, TNF-alfa enlazado a un polipéptido anti-TNF-alfa, una secuencia homóloga del mismo, una porción funcional del mismo o una porción funcional de una secuencia homóloga del mismo, pero en la ausencia de agente modulador agregado. Cavidades que muestran al menos 2 pliegues, preferiblemente 5 pliegues, más preferiblemente 10 pliegues y más preferiblemente 100 pliegues o más incrementados o disminuidos en enlace polipéptido TNF-alfa (por ejemplo) comparado al nivel de actividad en la ausencia del modulador, se seleccionan para análisis adicional.

La invención proporciona otros kits útiles para selección de moduladores del enlace del receptor TNF-alfa/TNF-alfa, así como kits útiles para diagnosis de trastornos caracterizado por disfunción de TNF-alfa. La invención también proporciona kits útiles para seleccionar moduladores de trastornos, así como kits para su diagnosis, los trastornos se caracterizan por uno o más procesos que involucran TNF-alfa. Kits útiles de conformidad con la invención pueden incluir un TNF-aislado. Alternativamente, o en adición, un kit puede comprender células transformadas para expresar TNF-alfa. En una modalidad adicional, un kit de conformidad con la invención, puede comprender un

polinucleótido que codifica TNF-alfa. En aún una modalidad adicional, un kit de conformidad con la invención puede comprender los cebadores específicos útiles para amplificación de TNF-alfa. Los kits útiles de conformidad con la invención pueden comprender un polipéptido TNF-alfa aislado, un homólogo del mismo, o una porción funcional del mismo. Un kit de conformidad con la invención puede comprende células transformadas para expresar el polipéptido. Los kits pueden contener más de un polipéptido. En una modalidad adicional, un kit de conformidad con la invención comprende un polinucleótido que codifica el TMF-alfa. En aún una modalidad adicional, un kit de conformidad con la invención puede comprender los cebadores específicos útiles para amplificación de una macromolécula tal como, por ejemplo, TNF-alfa. Todos los kits de conformidad con la invención comprenderán los artículos de estado o combinaciones de artículos y envasado de materiales de los mismos. Los kits también incluirán instrucciones para uso.

Además, será claro a una persona experta que puede ser posible "injertar" uno o más de los CDR mencionados anteriormente para los Nanocuerpos de la invención en otros "andamiajes", que incluye pero no se limita a andamiaje humano o andamiaje no se inmunoglobulina. Andamiaje adecuado y técnicas para el

1104

injerto CDR serán claros a la persona experta y son bien conocidos en la técnica, véase por ejemplo, los documentos US-A-7,180,370, WO 01/27160, EP 0 605 522, EP 0 460 167, US-A-7,054,297, Nicaise et al., Protein Science (2004), 13:1882-1891; Ewert et al., Methods, 2004 Oct; 34(2):184-199; Kettleborough et al., Protein Eng. 1991 Oct; 4(7): 773-783; O'Brien and Jones, Methods Mol. Biol. 2003: 207: 81-100, y Skerra, J. Mol. Regognit. 2000: 13: 167-187 y Saerens et al., J. Mol. Biol. 2005 Sep 23; 352(3): 597-607, y las referencias adicionales citadas en este documento. Por ejemplo, las técnicas conocidas per se para injertar CDR de rata o ratón en estructuras y andamiajes humanos se puede usar de una manera análoga para proporcionar proteínas quiméricas que comprenden uno o más de los CDR de los Nanocuerpos de la invención y una regiones de estructura humana o secuencias.

Así, en otra modalidad, la invención comprende un polipéptido quimérico que comprende al menos una secuencia CDR escogida del grupo que consiste de secuencias CDR1, secuencias CDR2 y secuencias CDR3 mencionadas en este documento para los Nanocuerpos de la invención. Preferiblemente, tal polipéptido quimérico comprende al menos una secuencia CDR escogida del grupo que consiste de las secuencias CDR3 mencionadas en este documento para los Nanocuerpos de la invención, y opcionalmente también al

menos una secuencia CDR escogida del grupo que consiste de las secuencias CDR1 y secuencia CDR2 mencionada en este documento para los Nanocuerpos de la invención. Por ejemplo, tal polipéptido quimérico puede comprender una secuencia CDR escogida del grupo que consiste de las secuencias CDR3 mencionadas en este documento para los Nanocuerpos de la invención, una secuencia CDR escogida del grupo que consiste de las secuencias CDR1 mencionadas en este documento para los Nanocuerpos de la invención y una secuencia CDR escogida del grupo que consiste de las secuencias CDR1 y secuencias CDR2 mencionadas en este documento para los Nanocuerpos de la invención. Las combinaciones de CDR que se mencionan en este documento como se prefiere para los Nanocuerpos de la invención usualmente también serán preferidas para estos polipéptidos quiméricos.

En los polipéptidos quiméricos, los CDR se pueden enlazar a secuencias de aminoácido adicional y/o pueden ser enlazados entre sí vía secuencias de aminoácido, en las secuencias de aminoácido son preferiblemente secuencias de estructura o son secuencias de aminoácido que actúan como secuencias de estructura, o juntos forman un andamiaje para presentar los CDR. Se hace nuevamente referencia a la técnica anterior mencionada en los últimos párrafos. De acuerdo a una modalidad preferida, las secuencias de

1100

aminoácido son secuencias de estructura humana, por ejemplo secuencias de estructura V_H3. Sin embargo, también se pueden usar secuencias de estructura, no humana, sintética, semi-sintética o no de inmunoglobulina. Preferiblemente, las secuencias de estructura usadas son de forma tal que (1) el polipéptido quimérico es capaz de enlazar xxxx, es decir con una afinidad que es al menos 1%, preferiblemente al menos 5%, más preferiblemente al menos 10%, tal como al menos 25% y hasta 50% o 90% o más de la afinidad del Nanocuerpo correspondiente de la invención; (2) el polipéptido quimérico es adecuado para uso farmacéutico; y (3) el polipéptido quimérico es preferiblemente esencialmente no inmunogénico bajo las condiciones planeadas para uso farmacéutico (es decir, indicación, forma de administración, dosis y régimen de tratamiento) del mismo (el cual puede ser esencialmente análogo a las condiciones descritas en este documento para el uso de los Nanocuerpos de la invención).

De acuerdos a una modalidad no limitante, el polipéptido quimérico comprende al menos dos secuencias CDR (como se menciona anteriormente) unidas vía al menos una secuencia de estructura, en la cual preferiblemente al menos una de las dos secuencias CDR es una secuencia CDR3, con la otra secuencia CDR siendo una secuencia CDR1 o CDR2. De conformidad a una modalidad preferida, pero no

limitante, el polipéptido quimérico comprende al menos dos secuencias CDR (como se menciona anteriormente) unido a al menos dos secuencias de estructura, en la cual preferiblemente al menos una de las tres secuencias CDR es una secuencia CDR3, con las otras dos secuencias CDR siendo secuencias CDR1 o CDR2, y preferiblemente usando una secuencia CDR1 y una secuencia CDR2. De acuerdo a una modalidad específicamente preferido, pero no limitante, los polipéptidos quiméricos tienen la estructura CR1'-CDR1-FR2'-CDR2-FR3'-CDR3-FR4', en la cual CDR1, CDR2 y CDR3 son como se definen en este documento para los CDR de los Nanocuerpos de la invención, y FR1', FR2', FR3' y FR4' son secuencias de estructura. FR1', FR2', FR3' y FR4' pueden en particular ser secuencias de Estructura 1, Estructura 2, Estructura 3 y Estructura 4, respectivamente, de un anticuerpo humano (tal como secuencias V_H3) y/o partes o fragmentos de tales secuencias de Estructura. También es posible usar partes o fragmentos de un polipéptido quimérico con la estructura FR1'-CDR1-FR2-FR3'-CDR3-FR4. Preferiblemente, tal como partes o fragmentos son de forma tal que cubren el criterio mostrado en el párrafo precedente.

La invención también se refiere a proteínas u polipéptidos que comprenden y/o esencialmente consisten de polipéptidos quiméricos, a ácido nucleicos que codifican

tales proteínas o polipéptidos; a métodos para preparar tales proteínas y polipéptidos; a células hospedantes que expresan o son capaces de expresar tales proteínas o polipéptidos; a composiciones, y en particular a composiciones farmacéuticas, que comprenden tales proteínas o polipéptidos, ácidos nucleicos o células hospedantes; y al uso de tales proteínas o polipéptidos, tales ácidos nucleicos, tales células hospedantes y/o tales composiciones, en particular para propósitos profilácticos, terapéutico y diagnóstico, tal como los propósitos profilácticos, terapéuticos o diagnóstico mencionados en este documento. Por ejemplo, tales proteínas, polipéptidos, ácidos nucleicos, métodos, células hospedantes, composiciones y usos pueden ser análogos a las proteínas, polipéptidos, ácidos nucleicos, métodos, células hospedantes, composiciones y usos como se describen en este documento para los Nanocuerpos de la invención.

También se notará que, cuando los Nanocuerpos de la invención contienen una o más secuencias CDR que las secuencias CDR preferidas mencionadas anteriormente, estas secuencias CDR pueden ser cualquier secuencia CDR adecuada (es decir, adecuada para por propósitos descritos en este documento) y/o estas secuencias CDR se puede obtener en cualquier manera conocida per se, por ejemplo, de Nanocuerpos (preferido), dominios V_H de anticuerpos

1109

convencionales (y en particular de anticuerpos humanos), anticuerpos de cadena pesada, anticuerpos de 4 cadenas convencionales (tal como anticuerpos de 4 cadenas humanos) u otras secuencias de inmunoglobulina dirigidas contra TNF. Tales secuencias de inmunoglobulina dirigidas contra xxxx se pueden generar en cualquier manera conocida per se, como será claro a una persona experta en la técnica, es decir, por humanización con TNF o por selección de una biblioteca adecuada de secuencias de inmunoglobulina con TNF, o cualquier combinación adecuada del mismo. Opcionalmente, esto puede ser seguido por técnicas tales como mutagénesis dirigida al sitio o al azar y/o otras técnicas para maduración por afinidad conocidas per se. Técnicas adecuadas para generar tales secuencias de inmunoglobulina serán claras a una persona experta, y por ejemplo incluyen técnicas de selección revisadas por Hoogenboom, Nature Biotechnology, 23, 9, 1105-1116 (2005). Otras técnicas para generar inmunoglobulinas contra un objetivo específico, incluyen por ejemplo, la tecnología Nanoclon (como por ejemplo, se describe en la solicitud de patente provisional estadounidense no prepublicada 60/648,922), así llamada tecnología SLAM (como por ejemplo, se describe en la solicitud de patente Europea 0 542 810), el uso de ratones transgénicos que expresan inmunoglobulinas humanas o las técnicas de hibridoma bien conocidas (véase por ejemplo,

Larrick et al, Biotechnology, Vol. 7, 1989, p. 934). Todas estas técnicas se pueden usar para generar inmunoglobulinas contra TNF, y los CDR de tales inmunoglobulinas se pueden usar en los Nanocuerpos de la invención, es decir, como se resume anteriormente. Por ejemplo, la secuencia de tal CDR se puede determinar, sintetizado y/o aislado, e insertado en la secuencia de un Nanocuerpo de la invención (por ejemplo, para reemplazar el CDR nativo correspondiente), todas las técnicas usadas, conocidas per se como aquellas descritas en este documento, o Nanocuerpos de la invención que contienen tales CDR (o ácidos nucleicos que codifican al mismo) se pueden sintetizar de novo, nuevamente usando las técnicas mencionadas en este documento.

La invención ahora se describe por medios de los siguientes ejemplos no limitantes, en los cuales las figuras se muestra:

Nanocuerpos TNFα Monovalentes

Figura 1: Alineación de secuencia de nanocuerpos TNFα humano

Figura 2: alineación de secuencia de nanocuerpos TNFα específico de albúmina de suero

Figura 3: Enlace de nanocuerpos TNFα específico de albúmina a albúmina de suero humano

Figura 4: Enlace de nanocuerpos TNFα específico

de albúmina para albúmina de suero rhesus

Figura 5: Enlace de nanocuerpos TNFα especifico de albúmina a albúmina de suero de ratón

Figura 6: Pureza de TNFα y nanocuerpos de albúmian de suero (SDS-PAGE)

Figura 7: Análisis de Manchado Western de TNFα y nanocuerpos de albúmina de suero

Figura 8: Enlace de nanocuerpos TNFα a TNFα humano (ELISA)

Figura 9: Enlace de nanocuerpos TNFα a TNFα rhesus (ELISA)

Figura 10: Ensayo del receptor-inhibición en Enberl de TNFα humano

Figura 11: Ensayo receptor-inhibición en Enbrel para TNFα rhesus

Figura 12: Enlace de nanocuerpos TNFα a TNFα humano (Biacore)

Figura 13: Enlace de nanocuerpos TNFα a TNFα rhesus (Biacore)

Figura 14: Enlace de nanocuerpos TNFα a Proteína A (Biacore)

Figura 15: Tratamiento por temperatura de TNFα y nanocuerpos de albúmina de suero (Manchado Western)

Figura 16: Estabilidad: Tratamiento por temperatura de nanocuerpos TNFα (ELISA)

Figura 17. Tratamiento por temperatura de nanocuerpos de albúmina de suero (Biacore)

Nanocuerpos TNFα Bivalentes

Figura 18: Pureza de nanocuerpos TNFα bivalentes (SDS-PAGE)

Figura 19: Análisis de Manchado Western de nanocuerpos TNFα bivalente

Figura 20: Ensayo receptor-inhibición en Enbrel para nanocuerpos TNFα bivalentes

Figura 21: Estabilidad: Tratamiento por temperatura de nanocuerpos TNFα bivalentes (ELISA)

Nanocuerpos TNFα monovalentes humanizados

Figura 22: Alineación de secuencia múltiple de nanocuerpos humanizados TNF1

Figura 23: Alineación de secuencia múltiple de nanocuerpos humanizados TNF2

Figura 24: Alineación de secuencia múltiple de nanocuerpos humanizados TNF3

Figura 25: Alineación de secuencia múltiple de nanocuerpos humanizados ALB1

Figura 26: Pureza de TNFα humanizado y nanocuerpos de albúmina de suero (SDS-PAGE)

Figura 27: Análisis de Manchado Western de TNFα

humanizado y nanocuerpos de albúmina de suero

Figura 28: Enlace de nanocuerpos TNFα humanizados a TNFα humano

Figura 29: Enlace de nanocuerpos de albúmina de suero humanizado a albúmina de suero humano

Figura 30: Estabilidad: Tratamiento por temperatura de nanocuerpos TNFα humanizados (ELISA)

Nanocuerpos TNFα trivalentes

Figura 31: Pureza de nanocuerpos TNFα trivalentes (SDS-PAGE)

Figura 32. Análisis de Manchado Western de nanocuerpos TNFα trivalente

Figura 33: Estabilidad: Tratamiento por temperatura de nanocuerpos TNFα trivalentes (ELISA)

Nanocuerpos TNFα monovalentes humanizados (segunda serie)

Figura 34: Alineación de secuencia múltiple de nanocuerpos humanizados TNF1

Figura 35: Alineación de secuencia múltiple de nanocuerpos humanizados TNF2

Figura 36: Alineación de secuencia múltiple de nanocuerpos humanizados TNF3

Figura 37: Alineación de secuencia múltiple de nanocuerpos humanizados ALB1

Figura 38. Pureza de nanocuerpos TNFα humanizados (SDS-PAGE)

Figura 39: Análisis de Manchado Western de nanocuerpos TNFα humanizados

Figura 40: Enlace de nanocuerpos TNFα humanizados a TNFα humano

Figura 41: Estabilidad: Tratamiento por temperatura de nanocuerpos TNFα humanizados (ELISA)

Figura 42: Análisis de TNF60 purificado en gel de SDS-PAGE teñido de plata (A) gel SDS-PAGE teñido de coomassie (B) y en análisis de manchado Western usando anti-NB (C) para detección

Figura 43: Cromatograma de exclusión de tamaño analítico de TNF60 en Superdex HR75

Figura 45: Curva de respuesta a la dosis obtenida en ensayo de citotoxicidad con TNF-alfa humano usando Nanobody™ TNF60 en comparación con Enbrel (Etanercept), Humira (Adalimumab) y Remicade (Infliximab)

Figura 46: Curva de respuesta a la dosis obtenida en ensayo de citotoxicidad con TNFα rhesus usando Nanobody™ TNF60 en comparación con Enbrel (Etanercept), Humira (Adalimumab) y Remicade (Infliximab)

Figura 47: Perfil de farmacocinética de TNF60 en ratones

Figura 48: Perfil de inmunogenicidad de TNF60 en

ratones

Figura 49: Análisis de TNF56-PEG40 purificado, TNF56-PEG60, TNF56-biotina, TNF55-PEG40, TNF55-PEG60 y TNF55-biotina en gel de SDS-PAGE teñido de coomassie

Figura 50: Análisis de TNF56-PEG40 purificado en gel SDS-PAGE usando teñido de plata (A) teñido coomassie (B) y en análisis de manchado Western usando anti-NB (C) para detección

Figura 51: Cromatograma de exclusión por tamaño analítico de TNF56-PEG40 en Superdex HR 75

Figura 52: Cromatograma de exclusión por tamaño analítico de TNF56-PEG40 en Superdex HR 200

Figura 53: Curva de respuesta a la dosis obtenida en ensayo de citotoxicidad con TNFα humano usando Nanobody™ TNF56-PEG40 y el Nanobody ™ de tipo nativo monovalente TNF1 en comparación con Enbrel (Etanercept), Humira (Adalimimab) y Remicade (Infliximab)

Figura 54: Curva de respuesta a la dosis obtenida en ensayo de citotoxicidad con TNFα Rhesus usando Nanobody™ TNF56-PEG40 en comparación con Enbrel (Etanercept), Humira (Adalimumab) y Remicade (Infliximab)

Figura 55: Análisis de farmacocinética de Nanobody™ TNF56-PEG40 bivalente pegilado y TNF56-PEG60 después de la administración intravenosa en ratones

Figura 56: análisis de farmacocinética de

Naobody™ 3E-3E-PEG200 bivalente pegilado, Nanobody™ 3E-3E-PEG40 bivalente pegilado y Nanobody™ 3E-3E-AR1 biespecífico después de la administración intravenosa en ratones

Figura 57: Perfil de inmunogenicidad de TNF56-PEG40 y TNF56-PEG60 en ratones

Figura 58: Eficacia de TNF60 en la prevención de poliartritis crónica en ratones

Figura 59: Eficacia de TNF60 en tratamiento terapéutico de poliartritis crónica en ratones

Figura 60: Efecto de Nanobody™ TNF60 que forma una eficacia en la prevención de poliartritis crónica en ratones

Figura 61: Alineación de secuencia de Nanobodies™ PMP1C2, 3E, 1A y 3G

Figura 62: Modelo molecular de TNF-60

Las tablas añadidas forman una parte integral de la presente especificación y son como siguen:

Nanocuerpos TNFα monovalentes

Tabla 8: Listado de secuencia de nanocuerpos TNFα

Tabla 9: Valores Koff de nanocuerpos TNFα humanos

Tabla 10: Homología de TNFα y nanocuerpos de albúmina de suero para secuencias línea germinal humana

Tabla 11: Niveles de expresión de TNFα y nanocuerpos de albúmina de suero

Tabla 12: ELISA enlazado a TNFα humano y rhesus

Tabla 13: ensayo receptor-inhibición de nanocuerpos TNFα

Tabla 14. Análisis Biacore de nanocuerpos TNFα

Tabla 15: Enlace de nanocuerpos TNFα a TNFα (valores K_D)

Tabla 16: Potencia de nanocuerpos TNFα para neutralizar TNFα humano (a) y rhesus (b)

Tabla 17: OD280nm de TNFα y nanocuerpos de albúmina de suero después del tratamiento por temperatura

Tabla 18: Potencia de nanocuerpos TNFα después del tratamiento por temperatura

Nanocuerpos TNFα Bivalentes

Tabla 19: Listado de secuencia de nanocuerpos TNFα bivalentes y secuencia enlazadoras

Tabla 20: Constructos de nanocuerpo TNFα bivalentes

Tabla 21: Niveles de expresión de nanocuerpos TNFα bivalente

Tabla 22: Ensayo receptor-inhibición de nanocuerpos TNFα bivalente

Tabla 23: Potencia de nanocuerpos TNFα para neutralizar TNFα humano (a) y rhesus (b)

Tabla 24: OD280nm de nanocuerpos TNFα bivalentes

Nanocuerpos TNFα monovalentes humanizados

Tabla 25: Listado de secuencia de TNFα monovalente humanizado y nanocuerpos de albúmina de suero

Tabla 26: Niveles de expresión de TNFα humanizado y nanocuerpos de albúmina de suero

Tabla 27: Potencia de nanocuerpos TNFα para neutralizar TNFα humano

Tabla 28: OD280nm de TNFα humanizado y nanocuerpos de albúmina de suero

Nanocuerpos TNFα Trivalentes

Tabla 29: Listado de secuencia de nanocuerpos TNFα trivalente

Tabla 30: Constructos de nanocuerpo TNFα trivalente

Tabla 31: Niveles de expresión de nanocuerpos TNFα trivalentes

Tabla 32: Niveles de potencia de nanocuerpos TNFα para neutralizar TNFα humano

Tabla 33: Enlace de nanocuerpos trivalentes a albúmina de suero (valores K_D)

Tabla 34: OD280nm de nanocuerpos TNFα trivalentes

Nanocuerpos TNFα monovalentes humanizados (segunda serie)

Tabla 35: Listado de secuencia de nanocuerpos TNFα monovalentes humanizados de segunda serie

Tabla 36: Niveles de expresión de nanocuerpos TNFα humanizados

Tabla 37: Potencia de nanocuerpos TNFα para neutralizar TNFα humano

Tabla 38: OD280nm de nanocuerpos TNFα humanizados

Tabla 39: Comparación de bioactividad de nanocuerpos

Tablas Adicionales

Tabla 40: Observación de oligonucleótidos usados en la formación de Nanobodies ™ trivalentes

Tabla 41: Observación de oligonucleótidos usados en la clonación de Nanobodies ™ trivalentes

Tabla 42: Valores EC50 obtenidos en ensayos de citotoxicidad usando Nanobody ™ TNF60 trivalente en comparación con controles comerciales (Enbrel, Remicade, Humira)

Tabla 43: Determinación de afinidad de TNF60 y TNF24 en albúmina de suero humano en Biacore.

Nd, no determinado.

1120

Tabla 44: Observación de oligonucleótidos usados en la formación de Nanobodies ™ bivalentes

Tabla 45: Valores EC50 obtenidos en ensayos de citotoxicidad usando Nanobodies ™ bivalentes en comparación con controles comerciales (Enbrel, Remicade, Humira)

Tabla 46: Resultados de estudios de fibroblasto derivados del sinovio

Tabla 47: Resultados de estudios bolsas de aire de murina

EJEMPLOS

Ejemplo 1. Identificación de nanocuerpos específicos de TNFα y albúmina de suero

Se identifican nanocuerpos antagonísticos usando llamas (*Llama glama*) inmunizado con TNFα humano por 6 inyecciones de 100 µg de la cotocina en intervalos semanales. Se realiza la selección usando ensayo en base a competencia, en el cual nanocuerpos individuales se analizan por su capacidad para inhibir en enlace de TNFα etiquetado a su receptor. Los nanocuerpos específicos de albúmina se identifican de una llama inmunizada con albúmina de suero humano. La selección de nanocuerpos individuales se realiza por ELISA usando albúmina de humano, rhesus y ratón, proporcionando un panel de

nanocuerpos de reacción cruzada con la albúmina de suero de varias especies.

Ejemplo 2: Análisis de secuencia de nanocuerpos aislados

Diferentes clases de nanocuerpos se identifican en el análisis de secuencia (Figura 1) usando una matriz de registro BLOSUM62 y un valor de similitud significante de corte de ≥ 60%, Clase I (PMP1 C2, PMP1 G11, PMP1 H6), Clase II (PMP1 G5, PMP1 H2, PMP3 G2), Clase IIb (PMP1 D2), Clase III (PMP3 D10, PMP5 F10). La Tabla 8 lista las secuencias de estos anticuerpos TNFα (SEC ID Nos: 52 a 60).

En base al análisis de secuencia (Figura 2) se identifican clases diferentes de nanocuerpos de albúmina de suero usando la matriz de registro BLOSUM62 y un valor de similitud significante de corte ≥ 60%. La tabla 8 lista las secuencias de estos nanocuerpos de albúmina de suero (SEC ID Nos: 61 a 67).

Ejemplo 3: Análisis Biacore

TNFα

Se caracteriza el enlace de nanocuerpos a TNFα por resonancia de plasmón de superficie en un instrumento Biacore 3000. El TNF de diferentes especies es covalentemente enlazado a la superficie del chip sensor CM5

vía amina acoplada hasta que se alcanzó un incremento de 250 unidades de respuesta. Se inactivaron grupos reactivos restantes. Se valoraron enlaces de nanocuerpo a una concentración (1 en 1,000 diluido). Cada nanocuerpo se inyecta por 4 minutos a una velocidad de flujo de 45 µl/min para permitir el enlace del antígeno enlazado al chip. Amortiguador de enlace sin nanocuerpo se envía al chip en la misma velocidad de flujo para permitir la disociación espontánea del nanocuerpo enlazado por 4 horas. Se calculan valores Koff de los gramos de sensor obtenidos para los nanocuerpos diferentes.

Se analizan de cada una clase de proteínas impurificadas de nanocuerpos en Biacore. Los datos Koff se listan en la Tabla 9.

Los nanocuerpos representativos de cada clase se retienen para análisis adicional en base al valor Koff. Se selecciona para la clase I PMP1C2 (TNF1), se selecciona PMP1G5 (TNF2) como representativo de la Clase II; PMP5F10 (TNF3) se selecciona como representativo de la Clase III.

Albúmina de suero

Se sometió a ensayo el enlace como se describe anteriormente excepto que se usaron 1 en 20 diluciones. Las figuras 3, 4 y 5 ilustraciones seleccionadas de nanocuerpos

1123

TNFα específicos de albúmina contra albúmina de suero humano, rhesus y ratón usando proteína impurificada.

Los nanocuerpos se marcan de conformidad con valores Koff, véase Tabla III abajo:

Tabla III

Clase	Humano	Rhesus	Ratón
C	PMP6A8	PMP6A8	PMP6B4
C	PMP6B4	PMP6B4	PMP6A8
B	PMP6A6	PMP6A6	PMP6A6
B	PMP6C1	PMP6C1	PMP6C1
A	PMP6G8	PMP6G8	PMP6G8
A	PMP6A5	PMP6A5	PMP6A5
D	PMP6G7	PMP6G7	PMP6G7

El mejor Koff se obtiene para elementos de la familia C y familia B. La reactividad cruzada entre albúmina de suero de ratón, humano y rhesus también se observó para elementos de aquellas familias. Un nanocuerpo representativo de clase B y C se define por análisis adicional: PMP6A6 (ALB1) se selecciona como representativo de Clase B y PMP6A8 (ALB2) se selecciona como representativo de Clase C.

Ejemplo 4: Clonación de nanocuerpos monovalentes en pAX051

Descripción de vector de expresión *Escherichia coli*

pAX051 es un derivado de pUC19. Contienen el promotor LacZ el cual es capaz de controlar la inducción de

expresión usando IPTG. El vector tiene un gen de resistencia por Ampicilina o Carbenicilina. Los sitios de clonación múltiple albergan varios sitios de restricción del cual *Sfi*I y *BstE*II son frecuentemente usados para clonación de Nanobodiest™. En estructura con la secuencia que codifica NB de los códigos del vector para una etiqueta c-myc C-terminal y una etiqueta (His)6. El péptido de señal es la secuencia líder gen3 la cual transloca el Nanobody™ expresado al periplasma.

La codificación de ADN para los nanocuerpos seleccionados TNF1 (PMP1C2), TNF2 (PMP1G2), TNF3 (PMP5F10), ALB1 (PMP6A6) y ALB2 (PMP68A), se clonaron en pAX051 y el constructo se transformó a células electropotentes TG1. Los clones fueron analizados por inserto de RCP y la secuencia de nucleótido se determinó de 4 clones positivos. Se prepararon soluciones base de glicerol a partir de clones que contienen la secuencia correcta y se almacenaron a -80°C.

Ejemplo 5: Expresión de nanocuerpos monovalentes

Se inició un precultivo inoculando una colonia única del clon que expresa los nanocuerpos respectivos a 37°C en caldo Luria, ampicilina/carbenicilina (100 µg/ml) y glucosa al 2% durante la noche. Este precultivo se usó para inocular. El inóculo es 1% por ciento (p/p) del cultivo de

1125.

producción (medio TB + ampicilina/carbenicilina + Glucosa al 0.1%). El cultivo de producción se hace crecer a 37°C hasta que se alcanza un OD600 nm de 5-10, y la expresión del nanocuerpo es inducida agregando IPTG (concentración final de 1 mM). La expresión de proteína se permite continuar ya sea por 4 horas a 37°C o durante la noche a 28°C, en tal punto, las células son colectadas por centrifugación y almacenadas como pasta celular húmeda a -20°C.

Los extractos periplásmicos preparativos de la pasta celular húmeda almacenados a -20°C, se hacen resuspendiendo la pelotilla en amortiguador Peri (50 mM de NaH_2PO_4, 300 mM de NaCl, ajustado a pH 8.0), rotando la mezcla por 30 minutos a 4°C y centrifugando la mezcla usando centrífuga preparativa (Sorvall RC-3C Plus con un rotor de H-6000A), para formar en pelotilla las células. El sobrenadante que representa un extracto robusto del espacio periplásmico, se colecta para purificación adicional.

Los nanocuerpos etiquetados con His(6), son purificados en Cromatografía de Afinidad Metálica Inmovilizada (IMAC). La resina TALO (Clontech), es procesada de conformidad con las instrucciones del fabricante. Los extractos son incubados con la resina por 30 minutos a TA en un rotator. La resina es lavada con PBS y transferida a una columna. La resina envasada es lavada

1126

con 15 mM de imidazol. Los nanocuerpos son eluidos de la columna usando 150 mM de imidazol. Las fracciones eluidas son analizadas por manchado en una membrana Hybond y visualización con Ponceau. Las fracciones que contienen la proteína, son combinadas y dializadas contra PBS. Las proteínas dializadas con colectadas, esterilizadas en filtro, determinadas de concentración y analizadas en alícuotas a -20°C.

Caracterización de nanocuerpos TNFα monovalentes

Ejemplo 6: Homología de secuencias de línea germinal humana

Las secuencias de aminoácido de nanocuerpos, fueron comparadas con las secuencias de línea germinal humana, como se representa en la Tabla 10. En orden de homología a las secuencias humanas, los nanocuerpos se clasifican como sigue: TNF1>TNF2>TNF3 para los nanocuerpos TNα; ALB1>ALB2 para los nanocuerpos de albúmina de suero.

Ejemplo 7: Nivel de expresión

Se calcularon los niveles de expresión y se representan en la Tabla 11. Para proporcionar la clasificación de nanocuerpos como sigue: TNF1>TNF2>TNF3 para los nanocuerpos TNFα; ALB1>ALB2 para los nanocuerpos de albúmina de suero.

1127

Ejemplo 8: Análisis SDS-Page

Para determinar la pureza, las muestras de proteína fueron analizadas en gel de SDS-PAGE al 15%. Se agregó amortiguador de muestra Laemmli 10 µl a 10 µl (1 µg) de proteína purificada, la muestra se calentó por 10 minutos a 95°C, se enfrió y cargó en gel de SDS-PAGE al 15%. El gel se procesó de conformidad con los procedimientos generales y se tiñó con Azul Brillante de Coomasie (CBB). La Figura 6 representa el SDS-PAGE para los nanocuerpos específicos de albúmina de suero y específicos de TNF-α.

Ejemplo 9: Análisis de manchado Western

Se cargaron 100 ng de proteína purificada en el gel. Después del SDS-PAGE, las proteínas fueron transferidas a una membrana de nitrocelulosa usando la Célula de Transferencia Electroforética Mini Trans-Blot® (Biorad). La membrana se bloqueó durante la noche en PBS, caseína al 1% a 4°C. Como todos los constructos se fusionaron a etiqueta c-myc, se usó el anticuerpo anti-myc monoclonal de ratón como una herramienta de detección. Además, se usó el anti-Nanocuerpo policlonal de conejo (R23) como una herramienta de detección. El manchado se incubó por 1 hora a temperatura ambiente con agitación en anticuerpo anti-myc diluido 1/2000 en PBS ó anticuerpo

anti-Nanocuerpo 1/200 en PBS, 1% de caseína. La membrana se lavó 5 veces en PBS antes de que se aplicara el anticuerpo secundario (conjugado de fosfatasa alcalina IgG de conejo-anti-ratón, Sigma, A1902, diluida 1/1000 en PBS o conjugado de fosfatasa alcalina IgG anti-conejo de cabra, Sigma, A8025, 1% de caseína). Después de la incubación con agitación suave por 1 hora a temperatura ambiente, la membrana se lavó 5 veces en PBS. Los manchados fueron revelados usando soluciones de BCIP/NBT y la reacción se detuvo lavando el manchado con agua milliQ cuando las bandas fueron claramente visibles. La Figura 7 representa el análisis de manchado Western.

Ejemplo 10: Enlace ELISA a TNFα Rhesus y humano

Se realizó un ELISA para examinar el enlace a TNFα humano y Rhesus. Se revistió una placa Maxisorp de 96 cavidades con 2 μg/ml de Neutravidina en PBS ON a 4°C. Las placas fueron bloqueadas con 1% de caseína por 2 horas a TA. El TNFα biotinilado (400 ng/ml), se agregó a las cavidades y se incubó por 1 hora a TA. Las muestras de nanocuerpos fueron diluidas partiendo a 2 μg/ml y usando diluciones 1 a 3. Los nanocuerpos fueron detectados usando fosfatasa alcalina anti-myc de ratón (1/2000 diluida) y anti-ratón (1/200 diluida, Sigma A1902) y pNPP (2mg/ml) como un sustrato. Las Figuras 9 y 10 representan el enlace

1129

en ELISA un TNFα Rhesus y humano.

Los resultados son resumidos en la Tabla 12. El TNF1 y TNF3, mostró enlace a TNFα tanto humano como Rhesus. El TNF2 es enlazado a TNFα humano, pero solamente es débilmente reactivo a TNFα Rhesus.

Ejemplo 11: Ensayo de inhibición del receptor

La capacidad para inhibir la interacción del ligando-receptor, se analizó para TNFα humano y Rhesus. Se revistió una placa Maxisorp de 96 cavidades con 2 μg/ml de Enbrel en PBS ON a 4°C. Las placas fueron bloqueadas con 1% de caseína por 2 horas a TA. Las muestras de nanocuerpos fueron pre-incubadas por 30 minutos a TA con TNFα biotinilado (10 ng/ml) partiendo a una concentración de 5 μg/ml y usando diluciones 1 en 2. Las muestras fueron agregadas a las placas e incubadas por 1 hora a TA. El TNFα biotinilado se detectó usando fosfatasa alcalina de Estravidina (1/2000 diluida) y pNPP (2 mg/ml) como sustrato. Las Figuras 11 y 12, representan una inhibición de ELISA para TNFα humano y Rhesus. Los resultados se resumen en la Tabla 13. La inhibición del enlace de ligando/receptor, se observa para TNF1 y TNF3, para TNF tano humano como Rhesus, mientras el TNF2 es solamente inhibición de TNFα humano.

1130

Ejemplo 12: Análisis Biacore

Enlace TNFα

Se realizó el análisis como se describe en el Ejemplo 3. Las Figuras 13 y 14 ilustran el enlace a TNFα Rhesus y humano vía análisis Biacore. Los resultados se resumen en la Tabla 14. Los experimentos de enlace en Biacore, confirman los resultados de ELISA: enlace reactivo cruzado para TNF1 y TNF3, mientras el TNF2 solamente se enlaza significantemente a TNFα humano.

Albúmina de suero

El enlace se ensayó como se describe anteriormente, excepto que se usó la serie de diferentes concentraciones. Cada concentración se inyectó por 4 minutos a una velocidad de flujo de 45 µl/min para permitir el enlace al antígeno unido al chip. El amortiguador de enlace sin analito, se asentó sobre el chip a la misma velocidad de flujo para permitir la disociación de nanocuerpo unido. Después de 15 minutos, el analito unido restante, se removió por inyección de la solución de regeneración (25 mM de NaOH).

A partir de los sensogramas obtenidos para las diferentes concentraciones de cada analito, se calcularon los valores KD vía afinidad de estado listo cuando se alcanzó el equilibrio.

Los resultados son resumidos en la Tabla 15. Se observó una reactividad cruzada para tanto ALB1 como ALB2. La afinidad más alta se observó para ALB2 en TNFα humano y Rhesus. Sin embargo, la diferencia en afinidad para albúmina de suero humano de ratón contra humano/Rhesus, es más pronunciada para ALB2 (factor 4000), mientras para ALB1, se observa una diferencia de un factor 12.

Ejemplo 13: Bio-ensayo

Se usó la línea de células de fibroblasto de ratón L929 sensibles a TNFα, para medir la reactividad anti-TNFα de los nanocuerpos seleccionados. A una concentración suficientemente alta de TNFα en el medio, es decir, la dosis citotóxica, las células L929 se sometieron a necrosis. La inhibición de interacción de TNF con su receptor, se determina preincubando una serie de diluciones de anticuerpo con una concentración citotóxica de TNFα antes de agregar la mezcla a las células. La presencia de actinomicina D en el medio, sensibiliza las células además, al TNFα, resultando en una sensibilidad incrementada del bioensayo para TNFα libre.

Las células L929 se hicieron crecer a cercana confluencia, se plaquearon en placas microtituladoras de 96 cavidades a 5000 células por cavidad y se incubaron durante la noche. La actinomicina D se agregó a las células a una

1132

concentración final de 1 μg/ml. Las diluciones seriales de los nanocuerpos a ser probados, se mezclaron con una concentración citotóxica de TNFα (concentración de ensayo final es 0.5 ng/ml o 15 IU/ml). Después de al menos, 30 minutos de incubación a 37°C, esta mezcla se agrega a las células plaqueadas. Las placas son incubadas por 24 horas a 37°C y CO_2 al 5%. La viabilidad celular se determina por el uso de la sal de tetrazolio WST-1. Las curvas de respuesta de dosis y los valores EC50, son calculados con el Prima Graphpad.

Los resultados son resumidos en la Tabla 16 para TNFα humano y Rhesus. Basados en su potencia para neutralizar la actividad citotóxica, las moléculas son clasificadas como sigue: TNF3>TNF1>TNF2 para TNFα humano y TNF1= TNF3>TNF2 para TNFα Rhesus.

Ejemplo 14: Enlace de Proteína A

La Figura 14 representa el enlace de Proteína A analizado en Biacore como se describe en el Ejemplo 12. Se obtuvo el enlace positivo para TNF1, TNF2, ALB1. No se observó ningún enlace o enlace débil para TNF3 y ALB2.

Ejemplo 15: Estabilidad de temperatura

Las muestras fueron diluidas a 200 μg/ml y divididas en 8 alícuotas que contienen 500 μl. Los viales

1132

diferentes fueron incubados cada uno a una temperatura dada variando desde TA hasta 90°C. Después del tratamiento, las muestras fueron enfriadas por 2 horas a TA, se mantuvieron a 4°C. Se removieron los precipitados por centrifugación por 30 minutos a 14,000 rpm. El SN fue cuidadosamente removido y analizado adicionalmente.

OD280nm

El OD a 280 nm, se midió y la concentración se calculó. Los resultados se resumen en la Tabla 17. Se observó un decremento en el contenido de proteína para TNF2 y TNF3 partiendo a 80°C, mientras que para ALB2, se observó un decremento partiendo de 70°C.

Manchado Western

2 μg de proteína tratada, se separaron en SDS-PAGE al 15% y se transfirieron a una membrana de nitrocelulosa y se trataron como se describe anteriormente. La detección se realizó usando anti-Nanocuerpo policlonal (R23, 1/2000 diluido), y peroxidasa picante de caballo anti-conejo (DAKO, P0448, 1/2000 diluida). La figura 15 representa el análisis de Manchado Western. Se observó una gota transparente en la concentración de proteína para ALB2 tratados a 70, 80 y 90°C. La agregación todavía se observó para TNF1 tratado a 70, 80, y 90°C; para TNF3 tratado a

90°C; para ALB1 tratado a 90°C, significa que todavía contiene rastros de precipitados, los cuales resultan en una lectura OD280nm superior. Esto explica porqué la concentración de la proteína medida a OD280 nm, no se reduce para TNF1, TNF3 y ALB1 tratados estas T superiores.

ELISA

El ELISA para detectar enlace a TNFα humano, fue esencialmente realizado como se describe en el Ejemplo 10. Los resultados se presentan en la Figura 16. El enlace de TNFα humano, fue reducido para TNF1, TNF26 y TNF3 partiendo a 80°C.

Bio-Ensayo

El bio-ensayo se realizó como se describe en el Ejemplo 13. Los resultados son resumidos en la Tabla 18. La potencia de los nanocuerpos se reduce para TNF1 partiendo a 70°C; para TNF2 y TNF3 partiendo a 80°C.

Biacore

El enlace a albúmina de suero humano se determinó como se describe en el Ejemplo 12. Se usó una concentración fija (1 en 50 diluida). Los resultados se presentan en la Figura 17. El tratamiento de temperatura no está influenciando el enlace a albúmina de suero para ALB1. El

tratamiento tiene un efecto en el Kon para ALB2 partiendo de T-70°C.

Nanocuerpos Bivalentes

Ejemplo 16: Formateado de nanocuerpos específicos de TNFα bivalentes

Se formatearon TNF1, TNF2 y TNF3 a nanocuerpos bivalentes. Se usó un espaciador entre los dos bloques de construcción ya sea un enlazador GlySer 9AA (Tabla 19 SEC ID NO:68), o un enlazador GlySer 30 AA (Tabla 19 SEC ID NO:69). Esto generó constructos representados por la Tabla 20. La Tabla 19 lista las secuencias de estos nanocuerpos TNFα bivalentes (SEC ID NOs: 70 a 75).

Ejemplo 17: Expresión de nanocuerpos específicos de TNFα bivalentes

Se realizó la expresión como se describe en el Ejemplo 5. Los nanocuerpos etiquetados con His(6), fueron purificados en Cromatografía de Afinidad Metálica Inmovilizada (IMAC). La resina Ni-NTA (Qiagen), fue procesada de conformidad con las instrucciones del fabricante. Los extractos fueron incubados con la resina e incubados por 30 minutos a TA en un rotator. La resina se lavó con PBS y se transfirió a una columna. La resina envasada se lavó con PBS (1 en 10 diluida). La columna fue

pre-eluida con 15 mM de Imidazol. Los nanocuerpos fueron eluidos de la columna usando 25 mM de ácido cítrico a pH 4. Las fracciones eluadas fueron analizadas por manchado en la membrana Hybond y por visualización con Ponceau. Las fracciones que contienen la proteína, fueron combinadas y además purificadas en un intercambio catiónico, seguido por exclusión de tamaño. Las proteínas purificadas fueron colectadas, esterilizadas en filtro, determinadas de concentración y almacenadas en alícuotas a -20°C.

Caracterización de nanocuerpos específicos de TNFα bivalentes

Ejemplo 18: Nivel de expresión

Se calcularon los niveles de expresión de los nanocuerpos TNFα bivalentes y se representan en la Tabla 21. El enlazador no tiene efecto significante en el nivel de expresión de los nanocuerpos.

Ejemplo 19: SDS-PAGE

Se realizó SDS-Page como se describe en el Ejemplo 8. La Figura 18 muestra el resultado del SDS-Page.

Ejemplo 20: Manchado Western

Se realizó análisis de Manchado Western como se describe en el Ejemplo 9. La Figura 19 representa los

resultados del Manchado Western.

Ejemplo 21: Ensayo de Inhibición del receptor

El ensayo se realizó como se describe en el Ejemplo 11. La Figura 20 y la Tabla 22, representan los resultados. El mejoramiento de la inhibición del enlace ligando/receptor, se observó para todos los nanocuerpos bivalentes, comparados con el formato monovalente.

Ejemplo 22: Bio-ensayo

El ensayo se realizó como se describe en el Ejemplo 13. Los resultados son resumidos en la Tabla 23. Basados en su potencia para neutralizar la actividad citotóxica, el TNF8, TNF7, TNF9 y TNF5, tienen una potencia en el intervalo de Enbrel.

Ejemplo 23: Estabilidad de temperatura

Se analizaron muestras como se describe en el Ejemplo 15.

OD280 nm

El OD a 280 nm, se midió y la concentración se calculó. Los resultados se resumen en la Tabla 24. Se observó un decremento en el contenido de proteína para TNF4 y TNF7 partiendo a 70°C, mientras que para TNF5, TNF6, TNF8

y TNF9, se observó un decremento partiendo de 80°C.

Manchado Western

Se analizaron muestras para determinar la presencia de agregados como se describe en el Ejemplo 15.

ELISA

El ELISA para detectar enlace a TNFα humano, fue esencialmente realizado como se describe anteriormente. Los resultados se presentan en la Figura 21. El enlace de TNFα humano, fue reducido para TNF5, TNF6, TNF8 y TNF9 partiendo a 80°C, para TNF4 y TNF7 partiendo de 70°C.

Nanocuerpos monovalentes humanizados

Ejemplo 24: Identificación de posiciones de aminoácido no humanos en TNFα y nanocuerpos específicos de albúmina de suero

La Figura 22 (TNF1), Figura 23 (TNF2), Figura 24 (TNF3) y Figura 25 (ALB1), representan alineaciones de secuencias múltiples (Clustal W 1.7), con secuencias DP51, DP53, DP54 y DP29.

Además de las mutaciones de aminoácido, la optimización de codón se realizó proporcionando las secuencias de la Tabla 25 SEC ID NOS: 76 a 89 (Nanocuerpos contra TNF-alfa y albúmina de suero humano,

respectivamente).

Ejemplo 25: Generación de mutantes de codón optimizado

Los oligonucleótidos fueron sintetizando abarcando la secuencia completa de los nanocuerpos.

Ejemplo 26: Expresión de nanocuerpos específicos de TNFα bivalentes

Se realizó la expresión como se describe en el Ejemplo 5.

Caracterización de nanocuerpos humanizados

Ejemplo 27: Nivel de expresión

La Tabla 26 representa los niveles de expresión calculados. La expresión se logró con rendimientos en el intervalo de 3.5-11.7 mg/ml. El tiempo de inducción no influencia el rendimiento.

Ejemplo 28: SDS-PAGE

Se realizó SDS-Page como se describe en el Ejemplo 8. La Figura 26 muestra el gel de SDS-Page.

Ejemplo 29: Manchado Western

Se realizó análisis de Manchado Western como se describe en el Ejemplo 9. La Figura 27 representa los

resultados del Manchado Western.

Ejemplo 30: Bio-ensayo

El ensayo se realizó como se describe en el Ejemplo 13.

Los resultados de los nanocuerpos humanizados son resumidos en la Tabla 27. Los nanocuerpos de tipo nativo son incluidos como referencia.

Ejemplo 31: Biacore

El análisis se realizó como se describe en el Ejemplo 12. La Figura 28 y 31, muestran los resultados de Biacore.

Ejemplo 32: Estabilidad de temperatura

Se analizaron muestras como se describe en el Ejemplo 15.

OD280 nm

El OD a 280 nm, se midió y la concentración se calculó. Los resultados se resumen en la Tabla 28.

No se observó un decremento significante en la concentración de proteína para los nanocuerpos humanizados TNF1 (TNF13-14). Se observó un decremento en la concentración de proteína para TNF2 humanizado (TNF15-19) y

TNF3 (TNF20-23), partiendo a 80°C. Se observó un decremento en la concentración de proteína para ALB1 humanizado (ALB4-5), partiendo a 70°C y para ALB3 partiendo a 60°C.

Manchado Western

Se analizaron muestras para determinar la presencia de agregados como se describe en el Ejemplo 15.

ELISA

El ELISA para detectar enlace a TNFα humano, fue esencialmente realizado como se describe en el Ejemplo 15. Los resultados se presentan en la Figura 30.

El enlace a TNFα humano es comparable para TNF1 WT tratado con temperatura y el TNF13 y 14 humanizado; para TNF2 WT tratado con temperatura y el TNF15-19 humanizado: el enlace a TNFα humano, es reducido por TNF21 y 22, y a una extensión menor para TNF23, mientras no se observa efecto para TNF20, comparado con el TNF3 WT tratado con temperatura.

Nanocuerpos trivalentes TNFα

Ejemplo 33: Formación de nanocuerpos específicos de TNFα trivalentes

Se formatearon TNF1, TNF2, TFN3, y ALB1 a nanocuerpos trivalentes. Como espaciador entre los 2

1192

bloques de construcción, se usó ya sea un enlazador GlySer 9AA (Tabla 19 SEC ID NO:68), o un enlazador GlySer 30 AA (Tabla 19 SEC ID NO:69). Esto genera los constructos de la Tabla 30. La Tabla 29 lista las secuencias de nanocuerpos TNFα trivalentes (SEC ID NOS: 91 a 94).

Ejemplo 34: Expresión de nanocuerpos específicos de TNFα trivalentes

Se realizó la expresión como se describe en el Ejemplo 5. Los nanocuerpos etiquetados His(6), son purificados en una Cromatografía de Afinidad Metálica Inmovilizada (IMAC). La resina Ni-NTA (Qiagen), es procesada de conformidad con las instrucciones del fabricante. Los extractos son incubados con la resina e incubados por 30 minutos a TA en un rotator. La resina es lavada con PBS y transferida a una columna. La resina envasada es lavada con PBS (1 en 10 diluida). Se pre-eluye con 15 mM de imidazol. Los nanocuerpos son eluidos de la columna usando 25 mM de ácido cítrico a pH =4. Las fracciones eluadas son analizadas marcando en una membrana Hybond y visualizando con Ponceau. Las fracciones que contienen la proteína son combinadas y además purificadas en intercambio catiónico, seguido por exclusión de tamaño. Las proteínas purificadas son colectadas, esterilizadas con filtro, determinada la concentración y almacenadas en

alícuotas a -20°C.

Caracterización de nanocuerpos específicos de TNFα/SA trivalentes

Ejemplo 35: Nivel de expresión

Los niveles de expresión fueron calculados y representados en la Tabla 31.

Ejemplo 36: Análisis de SDS-PAGE

Se realizó SDS-Page como se describe en el Ejemplo 8. La Figura 31 muestra el gel de SDS-Page.

Ejemplo 37: Análisis de Manchado Western

Se realizó análisis de Manchado Western como se describe en el Ejemplo 9. La Figura 32 representa el análisis de Manchado Western.

Ejemplo 38: Bio-ensayo

El ensayo se realizó como se describe en el Ejemplo 13.

Los resultados de los nanocuerpos bivalentes, son resumidos en la Tabla 32. Basados en su potencia para neutralizar la actividad citotóxica, las moléculas son igualmente potentes comparables con su potencia como

moléculas bivalentes.

Ejemplo 39: Enlace a Albúmina de Suero Humano

El enlace se ensayó como se describe anteriormente, excepto que se usaron series de concentraciones diferentes. Cada concentración se inyectó por 4 minutos a una velocidad de flujo de 45 μ/min, para permitir el enlace al antígeno unido al chip. Después, el amortiguador de enlace sin analito, se asentó sobre el chip a la misma velocidad de flujo para permitir la disociación de nanocuerpo unido. Después de 15 minutos, el analito unido restante, se removió por inyección de la solución de regeneración (25 mM de NaOH).

A partir de los sensogramas obtenidos para las diferentes concentraciones de cada analito, se calcularon los valores KD vía afinidad de estado listo cuando se alcanzó el equilibrio.

Los resultados son resumidos en la Tabla 33. Se observó una reducción en la afinidad para el enlazador ALB1 formateado, comparado con el ALB1 de tipo nativo. La afinidad sin embargo, todavía está en el intervalo de 7.2-14 nM.

Ejemplo 40: Estabilidad de temperatura

Se analizaron muestras como se describe en el

1145

Ejemplo 15.

OD280 nm

El OD a 280 nm, se midió y la concentración se calculó. Los resultados se resumen en la Tabla 34. Se observó un decremento en el contenido de proteína para TNF4, TNF27 y TNF28 partiendo a 60°C, mientras que para TNF25 y TNF26, partiendo de 70°C.

Manchado Western

Se analizaron muestras para determinar la presencia de agregados como se describe en el Ejemplo 15.

ELISA

El ELISA para detectar enlace a TNFα humano, fue esencialmente realizado como se describe anteriormente. Los resultados se presentan en la Figura 33. El enlace de TNFα humano, fue reducido para TNF4 y TNF27 partiendo de 60°C, y para TNF25, TNF26 y TNF28 partiendo a 70°C.

Nanocuerpos monovalentes humanizados (segunda ronda)

Ejemplo 41: Identificación de posiciones de aminoácido no humano en TNFα y nanocuerpos específicos de albúmina de suero

La Figura 34 (TNF1), Figura 35 (TNF2), Figura 36

1146

(TNF3) y Figura 37 (ALB1), representan alineaciones de secuencias múltiples (Clustal W 1.7), con secuencias DP51, DP53, DP54 y DP29. Las moléculas mutadas fueron expresadas y purificadas como se describe anteriormente, proporcionando las secuencias de la Tabla 35, SEC ID NOs: 95 a 104 (contra TNF-alfa y albúmina de suero humano, respectivamente).

Caracterización de nanocuerpos humanizados

Ejemplo 42: Nivel de Expresión

La tabla 36 representa los niveles de expresión calculados. La expresión se logó con rendimientos en el intervalo de 0.5-2.7 mg/ml.

Ejemplo 43: SDS-PAGE

Se realizó SDS-Page como se describe en el Ejemplo 8. La Figura 38 muestra el gel de SDS-Page.

Ejemplo 44: Manchado Western

Se realizó análisis de Manchado Western como se describe en el Ejemplo 9. La Figura 39 representa los resultados del Manchado Western.

Ejemplo 45: Bio-ensayo

El ensayo se realizó como se describe en el

Ejemplo 13.

Los resultados de los nanocuerpos humanizados son resumidos en la Tabla 37. Los nanocuerpos de tipo nativo y la primera ronda de nanocuerpos humanizados, son incluidos como referencia.

Ejemplo 46: Biacore

El análisis se realizó como se describe en el Ejemplo 12. La Figura 40, muestra los resultados de Biacore.

Ejemplo 47: Estabilidad de temperatura

Se analizaron muestras como se describe en el Ejemplo 15.

OD280 nm

El OD a 280 nm, se midió y la concentración se calculó. Los resultados se resumen en la Tabla 38.

No se observó un decremento significante en la concentración de proteína para los nanocuerpos humanizados TNF1 (TNF29-30). Se observó un decremento en la concentración de proteína para TNF2 humanizado (TNF31-32) y TNF3 (TNF33), partiendo a 80°C.

1148

Manchado Western

Se analizaron muestras para determinar la presencia de agregados como se describe en el Ejemplo 15.

ELISA

El ELISA para detectar enlace a TNFα humano, fue esencialmente realizado como se describe en el Ejemplo 15. Los resultados se presentan en la Figura 41.

El enlace a TNFα humano es comparable para TNF1 WT y el TNF29 y TNF30 humanizado; comparable para TNF2 WT y el TNF31 y TNF32; y también para TNF3 WT y TNF33 humanizado.

Ejemplo Comparativo

En este Ejemplo Comparativo, se compararon nueve Nanocuerpos de la invención con tres Nanocuerpos del documento WO 04/041862, llamados "V_{HH}#1A" ó "1a", "V_{HH}3E" ó "3E" y "V_{HH}#3G" o "3G", respectivamente (SEC ID NOS: 1, 4 y 5 en el documento WO 04/041862). El ensayo usado como el ensayo a base de célula usando células KYM es referido en el documento WO 04/41862 (véase por ejemplo, el Ejemplo 1 bajo 3)). Los resultados son mencionados en la Tabla 39 siguiente. Como se puede ver, los Nanocuerpos de la invención tienen un valor EC50 en este ensayo, que es 18 veces mejor que el valor EC50 de 3E, el mejor desempeño de

Nanocuerpo de conformidad con el documento WO 04/041862.

Ejemplo 48: Generación de Nanobodies™ (Nanocuerpos) humanizados biespecíficos trivalentes

Los Nanocuerpos biespecíficos trivalentes, se formatearon y clonaron en el vector de expresión de E. coli pAX054 primero, y después se rescataron a través de RCP y se clonaron en el vector de expresión pPICZαA.

Descripción del vector de expresión de Escherichia coli

El pAX54, es un derivado de pUC19. Contiene el promotor LacZ el cual permite una inducción controlada de expresión usando IPTG. El vector tiene un gen de resistencia para Ampicilina o Carbenicilina. Los sitios de clonación múltiple, albergan varios sitios de restricción de los cuales, *Sfi*I y *BstE*II, son frecuentemente usados para clonación de los Nanobodies™. La señal peptídica es la secuencia líder gen3, la cual transloca el Nanobody™ expresado en el periplasma.

Descripción del vector de expresión de Pichia pastoris

El pPICZαA contiene un origen derivado de pUC de replicación, permitiendo la propagación en *E. coli*. Contiene el promotor del gen AOX1 de Pichia pastoris (oxidasa de alcohol 1). Esta región promotora de 942 bp (i) permite la expresión de alto nivel, inducible por metanol

del gen de interés, y (ii) dirige la integración del plásmido con el sitio *AOX1*, después de la transformación de Pichia con el ADN vector que es linearizado dentro de la región promotora de *AOX1* en 5'. Se nota que los vectores pPICZα, no contienen un origen de levadura de replicación y que, consecuentemente, los transformantes pueden solamente ser aislados si ocurre la recombinación entre el plásmido y el genoma de Pichia. El vector especifica la resistencia al antibiótico Zeocina en células hospederas tanto en *E. coli* como en *Pichia pastoris*. El vector incorpora la señal de secreción del factor de α-apareamiento de *Saccharomyces cerevisiae* permitiendo la secreción eficiente de la mayoría de las proteínas al medio de cultivo. El ATG de en la secuencia de señal del factor-α corresponde al ATG de iniciación nativo del gen *AOX1*. El sitio de clonación múltiple alberga varios sitios de restricción de los cuales, el XhoI/EcoRI o XhoI/NotI, son típicamente usados para fusión de las secuencias de codificación de Nanobody™ con la señal de secreción. El sitio de clonación múltiple es seguido por la región de terminación de la transcripción *AOX1*. Más detalles en este vector de expresión se pueden encontrar en el sitio de red de Invitrogen. (http://www.invitrogen.com/content/sfs/manuals/ppiczalpha_man.pdf)

Formateado de Nanocuerpos trivalentes

Se establecieron tres reacciones de RCP separadas, para amplificar la subunidad de Nanobody™ N-terminal, media y C-terminal, usando las combinaciones de oligo indicadas en el WPA-0012. El Nanobody™ N-terminal se amplificó usando M13_rev/ Rev_9GlySer_L108; el Nanobody™ medio se amplificó usando For_GlySer/Short y Rev_15BspEI_L108; el Nanobody™ C-terminal se amplificó usando For_BspEI/M13_for. Una reacción de RCP de 1 µl de ADN plásmido (50-100 ng), 1.5 µl de cebador delantero (10 µM → 300 nM), 1.5 µl de cebador inverso (10 µM → 300 nM), 1 µl dNTPs (10 mM → 0.2 mM), 5 µl de amortiguador (10x → 1x), 0.75 µl de enzima (3.5 U/µl → 2.6 U/µl) y 39.25 µl de H_2O con un volumen total de 50 µl, se preparó. Las secuencias cebadoras se proporcionan en la Tabla 40. Se inició un programa de RCP con 2 minutos a 94°C. Se repitió un ciclo de 30 segundos a 94°C, 30 segundos a 50°C y 1 minuto a 72°C, 30 veces y se siguió por 10 minutos a 72°C. La amplificación se verificó separando 5 µl de la reacción de RCP en gel de agarosa al 2%. El producto de RCP se purificó usando un Kit de Purificación de RCP QIAquick, de conformidad con las instrucciones del fabricante. Se usó una columna y se eluyó con 50 µl de amortiguador EB. El fragmento VHH N-terminal se preparó incubando 50 µl de DNA y 2 µl de BamHl (10 U/µl) en el amortiguador apropiado

recomendado por el fabricante a 37°C por 2 horas. Subsecuentemente, se agregaron 2 µl de SfiI (10U/µl) y la mezcla se incubó a 55°C por 2 horas. El fragmento de VHH medio se preparó incubando 50 µl de DNA y 2 µl de BamH1 (10 U/µl) y 2 µl de BspEl (10 U/µl) en el amortiguador apropiado recomendado por el fabricante a 37°C por horas. El fragmento VHH C-terminal se preparó incubando 50 µl de ADN con 2 µl de BspEI (10 U/µl) en el amortiguador apropiado recomendado por el fabricante a 37°C por 2 horas. Subsecuentemente, se agregaron 2 µl BstEII (10 U/µl) y la mezcla se incubó a 60°C por 1 hora. Las reacciones de digestión previas se separaron en gel de agarosa al 2%. Las bandas de VHH (350-450 pb), se cortaron por el gel y el ADN se purificó usando el Kit de Extracción de Gel QIAquick, de conformidad con las instrucciones del fabricante. Se usó una columna (con un máximo de 400 mg de gel de agarosa por columna) y el ADN unido se eluyó con 50 µl de amortiguador EB. La concentración de ADN se determinó midiendo el OD_{260} (1 unidad de OD = 50 µg/ml). Una mezcla de ligación con un volumen final de 10 µl que contiene 100 ng de vector pAX54, 12 ng de VHH N-terminal, 12 ng del fragmento VHH medio, 12 ng del fragmento VHH C-terminal, 1 µl de amortiguador de ligación y 1 µl de ligasa (3U), se preparó e incubó por 2 horas a temperatura ambiente. La transformación de *E. coli*, TG1, se realizó usando 2 µl de la mezcla de ligación. Las

colonias se analizaron usando RCP como se describe en el documento WPA-0010. El análisis de secuencia se realizó en clones positivos. La preparación del plásmido se realizó usando un Kit Miniprep de centrifugación Qiaprep (Qiagen), de conformidad con las instrucciones del fabricante y se describe anteriormente. El secuenciamiento se realizó en las instalaciones de secuencias VIB, Antwerp, Bélgica.

Amplificación de ADN de codificación

La región de codificación de Nanobody^{tm} clonada en el vector de expresión de E. coli pAX054, es rescatada a través de PCR usando un par cebador apropiado. Para asegurar que el Nanobody™ es expresado con un N-término nativo, la región de codificación es clonada en estructura con el sitio de desdoblamiento de Kex2 de la señal de secreción. El cebador delantero fusiona la parte C-terminal de la señal de secreción, hasta el sitio de reconocimiento de Xho1, con la región de codificación de Nanobody™. Se preparó una reacción de RCP de 1 μl de ADN de plásmido (50-100 ng), 1.5 μl de cebador delantero (10 μM → 300 nM), 1.5 μl de cebador inverso (10 μM → 300 nM), 1 μl de dTNPs (10 mM → 0.2 mM), 5 μl de amortiguador (10x → 1x), 0.75 μl de enzima (3.5 U/μl → 2.6 U) y 39.25 μl de H_2O con un volumen total de 50 μl. Las secuencias cebadoras se proporcionan en la Tabla 41. Se inició un programa de RCP con 2 minutos a

94°C. Un ciclo de 30 segundos a 94°C, 30 segundos a 50°C y 2 minutos a 72°C, se repitió 20 veces y se siguió por 10 minutos a 72°C. La amplificación se verificó por separación de 5 µl de la reacción de RCP en gel de agarosa al 2%. El producto de RCP se purificó usando el Kit de Purificación de PCR QIAquick, de conformidad con las instrucciones del fabricante. Se usó una columna y el ADN unido se eluyó con 50 µl de amortiguador EB.

Estrategia de clonación

El fragmento de ADN que codifica para el NB, así como también el vector de expresión de pPICZαA, es digerido con la enzima de restricción apropiada (XhoI + NotI). El inserto es obtenido incubando 50 µl del producto de PCR con 2 µl de XhoI (10 U/µl) y 2 µl de NotI (10 U/µl) en el amortiguador apropiado recomendado por el fabricante por 3 horas a 37°C. El vector es obtenido de manera similar, adaptando la cantidad de enzimas de restricción con la cantidad de plásmido. Tanto el vector como el fragmento de codificación de NB, son purificados y la concentración de ADN es cuantificada usando el BioPhotometer (Eppendorf). El fragmento y el vector aceptor son ligados en una relación equimolar usando 1 Unidad de ligasa T4 (Promega) por 30 minutos, a temperatura ambiente o durante la noche a 16°C. El ADN (20-30 ng), es transformado a células TG1. Las

colonias son analizadas a través de RCP usando los cebadores 3'AOX1 R y 5'AOX1 F. El análisis de secuencia es realizado en clones positivos. Se formateó TNF30, TNF33 y ALB8 a Nanobodies™ biespecíficos trivalentes. Se usó un espaciador entre los bloques de construcción de un enlazador GlySer 9AA.

Transformación de P. pastoris

Para aislar el plásmido de ADN, se inició un precultivo inoculando una colonia única del clon en 50 ml de Caldo Luria + Ampicilina o Carbenicilina (100 μg/ml) + 2% de glucosa e incubación a 37°C durante la noche. El ADN de plásmido es preparado usando el Kit Midi Plasmid (Qiagen), de conformidad con las instrucciones del fabricante. El ADN es linearizado incubando 30 μg de ADN de plásmido con 6 μl de BstX1 (10 U/μl) en el amortiguador apropiado, de conformidad con las instrucciones del fabricante por 3 horas a 45°C. El ADN digerido es purificado usando el kit de Purificación de PCR (Qiagen), de conformidad con las instrucciones del fabricante. El ADN es concentrado usando precipitación de EtOH de conformidad con los procedimientos estándares. Las células electrocompetentes X-33 son transformadas con 10 μg de ADN linearizado y las células se dejan crecer por 48 horas en una placa de agar YPD selectiva que contiene Zeocina

(100/250/500 µg/ml). La X-33 es una cepa de *Pichia pastoris* tipo nativa; la cepa misma, así como también las cepas recombinantes derivadas, contienen el gel nativo AOX1 y son capaces de metabolizar el metanol (Mut^+).

Los clones son seleccionados por nivel de expresión inoculando las colonias únicas en 1 ml de BGCM en una placa de 24 cavidades y se hicieron crecer por 48 horas a 30°C a 12 rpm. Las células son centrifugadas y se agrega BMCM fresco a las células para crecer a 30°C a 120 rpm durante 48 horas. Después, se agrega MeOH a una concentración final de 0.5% y las células se hacen crecer a 30°C a 120 rpm durante 8 horas, después de lo cual, se agrega MeOH nuevamente a una concentración final de 0.55. Las células se hacen crecer durante la noche a 30°C a 120 rpm. Las células son centrifugadas y el sobrenadante es colectado y analizado en ELISA como se describe en el Ejemplo 10.

Ejemplo 49: Expresión y purificación de Nanobodies™ humanizados biespecíficos trivalentes

Producción en Pichia pastoris

La composición de amortiguadores, soluciones y otros, puede ser encontrada en el sitio de red de Invitrogen

(http://www.invitrogen.com/content/sfs/manuals/ppiczalpha_man.pdf).

Se inició un precultivo inoculando una colonia única de la placa en 5 ml de YPD. El cultivo se hizo crecer durante la noche a 180 rpm y a 30°C. Al siguiente día, el pre-cultivo se diluyó a 50 ml de YPD y se hizo crecer durante la noche a 180 rpm y a 30°C. Los cultivos de producción se iniciaron inoculando el pre-cultivo a un OD600nm = 0.04-0-08 final. Los cultivos se hicieron crecer en BGCM por 24 horas a 30°C a 180 rpm y se centrifugaron a 4,500 rpm por 30 minutos. Las células se resuspendieron en 1/3 del volumen original en medio BMCM con un OD600 nm = 15-20 final. Las células se indujeron con MeOH a puntos de tiempo regulares, típicamente 3 veces/día, nunca excediendo el contenido de MeOH al 1%. Después de 50 horas de inducción, el sobrenadante es colectado.

Purificación de Nanocuerpo expresado en Pichia pastoris

El sobrenadante de cultivo se filtra sobre una membrana de micro filtración de .22 μm (Hydrosart, Sartorius). La muestra es concentrada usando diafiltración en una membrana de ultra filtración de 10 kDa (HydroSart, Sartorius) y se concentra a 0.5-1 L. Los Nanobodies™, son purificados usando cromatografía de afinidad de Proteína A (MabSelect Xtra, GE Healthcare), usando PBS como

amortiguador de corrida y Glicina [100 mM pH 2.5] para elución. Las muestras son analizadas usando 1.5 M de Tris pH = 8.8. Los Nanobodies™ son además procesados en Cromatografía de Intercambio Aniónico (Source 30Q, GE Healthcare). Las muestras son diluidas 10 veces con 10 mM de Piperazina a pH 10.2 y ajustadas a pH =10.2, con 1 M de NaOH y una conductividad de <2 mS/cm con agua MiliQ.

Los nanocuerpos son procesados en cromatografía de exclusión de tamaño (Superdex 75 pg, Hiload XK26/60, GE Helathcare) y se remueve el LPS vía Cromatografía de Intercambio Aniónico (Source 30Q, GE Healthcare), por pasos a través de una columna de 5 ml, la cual es sanitizada con NaOH 1 M y equilibrada en Dulbecco PBS.

Para determinar la pureza, las muestras de proteína son analizadas en gel de SDS-PAGE al 15% como se describe en el ejemplo 8. El gel es procesado usando SiverQuest™, de conformidad con los procedimientos generales descritos por el fabricante (Invitrogen). Alternativamente, el gel es procesado usando azul brillante de coomassie o en manchado western como se describe en el ejemplo 8 y 9. Los resultados se dan en la Figura 42.

Ejemplo 50: Caracterización de Nanobodies™ humanizados biespecíficos trivalentes

El TNF60 consiste de 363 aminoácidos. La proteína

tiene un peso molecular de 38,441 DA. El pI es 8.71. El coeficiente de extinción a 280 nm es 1.736.

Espectofotometría de masas

La masa de la proteína se determina en ESI-EM de conformidad con los procedimientos estándares. La masa teórica de TNF60 es 38,441 Da. La proteína tiene 2 puentes S-S, los cuales deben resultar en una masa de 38,435 Da En ESI-EM. La masa que fue experimentalmente determinada por TNF60 derivado de 3 intervalos de lotes diferentes de 38,433 Da hasta 38,435 Da, difiere máximamente 0.005% con la masa teórica.

Secuenciamiento N-terminal

El secuenciamiento N-terminal se realizó por degradación Edman de conformidad con los procedimientos estándares. El secuenciamiento N-terminal muestra que la secuencia de proteína para los primeros 7 aminoácidos es como sigue: EVQLVES. Esto es consistente con la secuencia de proteína teórica, lo cual indica el procesamiento N-terminal apropiado.

Clasificación Analítica

Las muestras (100 ug) se analizaron en la columna Superdex75 de alta resolución, para caracterizar los

diferentes lotes de Nanobody™. La cromatografía de exclusión de tamaño del Nanocuerpo, típicamente proporciona un pico simétrico, con un tiempo de retención de 11.5 ml en Superdex75. La absorbancia es típicamente registrada a 280, 254 y 214 nm. La medición de 214 nm permite sensibilidad de detección superior. El tamaño analítico en PBS, proporciona un pico simétrico. No se observan contaminantes. El tiempo de retención observado para 3 lotes diferentes es 11.5-11.55 ml. Se muestra un perfil representativo en la Figura 43.

Ejemplo 51: Enlace de TNF60 a TNFα humano en ELISA

La funcionalidad de TNF60, es decir, el enlace a TNFα humano, se analizó en ELISA como se describe en el ejemplo 10. Los resultados son resumidos en la Figura 44 y demuestran claramente un enlace saturable y dependiente de la dosis de 2 lotes de TNF60 a TNFα humano.

Ejemplo 52: Funcionalidad en ensayo a base de célula

La potencia para neutralizar la actividad citotóxica de TNFα, se analizó en un ensayo a base de célula como se describe en el ejemplo 13. Los resultados son resumidos en la Tabla 42 y en las Figuras 45 y 46.

Los datos muestran que el TNF60 tiene potencia en el intervalo de Enbrel/Etanercept y una potencia 10 veces mejor que Humira/Adalimumab y Remicade/Infliximab.

Ejemplo 53: Enlace de TNF60 a albúmina de suero

El enlace a albúmina de suero Rhesus y humano, se analizó en Biacore como se describe en el Ejemplo 12. Los valores KD, kon y koff, son representados en la Tabla 43. El TNF60 es comparado con TNF24, el cual es el Nanobody™ precursor biespecífico trivalente con bloques de construcción de tipo nativo.

La afinidad de TNF60 para albúmina de suero Rhesus y humana es similar. La afinidad es 2 veces inferior, comparada con la afinidad observada para TNF24, el cual es el tipo análogo de TNF60. El Kon es idéntico para ambas moléculas, pero el Koff es 2 veces superior para TNF60.

Ejemplo 54: Análisis de inmunogenicidad y farmacocinética de Nanocuerpos humanizados biespecíficos trivalentes en ratones

Animales

Los ratones DBAI o BALBc fueron calentados bajo una lámpara infrarroja y se inyectaron intravenosamente 200 µl de Nanobody™ (100 µg por ratón) en la cola. Las muestras de sangre de obtuvieron a puntos de tiempo diferentes y haciendo una pequeña incisión en la cola y colectando la sangre en un microtubo. Típicamente, el

sangrado se muestreó a t = 15 min, 2 horas, 4 horas, 6 horas, 1 día, 2 días, 3 días, 4 días, 7 días, 14 días. El suero se preparó de conformidad con los procedimientos estándares.

Determinación de la concentración de Nanobody™ (nanocuerpo) en suero de ratón

Se revistió un microtitulador de placa (NUNC, Maxisorb), con 2 µg/ml de neutravidina durante la noche a 4°C. La placa se lavó 5 veces con PBS/0.05% de Tween-20 y se bloqueó por 2 horas a TA con PBS/caseína al 1%. El TNFα humano biotinilado (1/2000 en PBS/0.2% de caseína; 400 ng/ml), se aplicó a las cavidades y se incubó por 1 hora a TA. El Nanobody™ de referencia estándar se aplicó iniciando a una concentración de 5 µg/ml y usando diluciones de 5 veces en PBS que contiene plasma de ratón al 1%. Los Nanobodies™ se dejaron enlazar por 2 horas a TA. La placa se lavó 5 veces y se aplicó anti-Nanocuerpo policlonal de conejo (R23) a una dilución de 2000 veces por una hora a TA. Después de lavar la placa, el enlace se detectó con anti-conejo-HRP policlonal de cabra (DAKO) a una dilución de 3000 veces por una hora a TA, y se tiñó con ABTS/H_2O_2. El OD405 nm fue medido.

Primero se usó ELISA para determinar el intervalo lineal de la referencia estándar. En un segundo ELISA, la

referencia estándar se usó a concentraciones en el intervalo lineal y típicamente usando diluciones de 2 veces. En este segundo ELISA, las muestras de prueba de suero fueron diluidas 100 veces y además, se hicieron diluciones de 5 veces en plasma de ratón al 1%, para determinar la dilución en la cual la muestra de suero proporciona una lectura en el intervalo lineal de la curva estándar. En un tercer ELISA, las muestras de suero son diluidas a una concentración apropiada determinada en el segundo ELISA y usando diluciones de 2 veces para determinación exacta de la concentración de Nanobody™ en las muestras de suero.

Se realizaron experimentos para determinar el perfil de farmacocinética de TNF60 en ratones (n=3). Se alcanzó un valor Cmax de 103,84 ± 31 µg/ml 15 minutos después de la administración. La vida media (t1/2β) por ser 1.9 días, similar a la vida media de albúmina de suero de ratón, indicando que TNF60 adopta la vida media de albúmina de suero. Los datos son representados en la Figura 47.

Determinación de anticuerpos anti-Nanocuerpos en ratones

Se revistió Nanobody™ a 5 µg/ml en PBS a 4°C durante la noche. La placa se lavó 5 veces con PBS/0.05% de Tween-20 y se bloqueó por 2 horas a TA con PBS/1% de caseína. Las muestras de suero fueron diluidas 100 veces y

aplicadas a las cavidades para incubación durante 1 hora a temperatura ambiente. La detección se realizó usando anti-ratón-HRP de conejo policlonal (DAKO, P0260) y usando ABTS como sustrato.

Las muestras de suero fueron diluidas 50 veces y analizadas por la presencia de anticuerpos anti-TNF60 de ratón. La carencia de inmunogenicidad se demostró para TNF60. Los datos son representados en la Figura 48.

Ejemplo 55: Generación de Nanobodies™ humanizados de vida media prolongada bivalentes

Descripción del vector de expresión de Pichia pastoris

Véase ejemplo 48

Formateado de Nanobodies™ bivalentes

Se establecieron dos reacciones de PCR separadas para amplificar la subunidad de Nanobody™ N-terminal y C-terminal usando los procedimientos como se indica en el WPA-0011. Para la amplificación del Nanobody™ N-terminal, se usaron PiForLong y Rev_30GlySer_L108 como combinación de cebador; para la amplificación del Nanobody™ C-terminal se usaron For_GlySer y PiRevCys1hum o alternativamente, For_GlySer y PiRevCys2hum, introduciendo los sitios de restricción requeridos para formatear y liberar residuos de cisteína requeridos para modificaciones C-terminal.

La reacción de RCP de 1 µl de ADN plásmido (50-100 ng), 1.5 µl de cebador delantero (10 µM → 300 nM), 1.5 µl de cebador inverso (10 µM → 300 nM), 1 µl dNTPs (10 mM → 0.2 mM), 5 µl de amortiguador (10x → 1x), 0.75 µl de enzima (3.5 U/µl → 2.6 U/µl) y 39.25 µl de H_2O con un volumen total de 50 µl, se preparó. Las secuencias cebadoras se proporcionan en la Tabla 44. Se inició un programa de RCP con 2 minutos a 94°C. Se repitió un ciclo de 30 segundos a 94°C, 30 segundos a 50°C y 1 minuto a 72°C, 30 veces y se siguió por 10 minutos a 72°C. La amplificación se verificó separando 5 µl de la reacción de RCP en gel de agarosa al 2%. El producto de RCP se purificó usando un Kit de Purificación de RCP QIAquick, de conformidad con las instrucciones del fabricante. Se usó una columna y se eluyó con 50 µl de amortiguador EB. El fragmento VHH N-terminal se preparó incubando 50 µl de DNA y 2 µl de BamHl (10 U/µl) en el amortiguador apropiado recomendado por el fabricante a 37°C por 1.5 horas. El fragmento de VHH C-terminal, se preparó incubando 50 µl de DNA y 2 µl de BamHl (10 U/µl) y 2 µl de EcoRI (10 U/µl) en el amortiguador apropiado recomendado por el fabricante a 37°C por horas. Las reacciones de digestión previas se separaron en gel de agarosa al 2%. Las bandas de VHH (350-450 pb), se cortaron por el gel y el ADN se purificó usando el Kit de Extracción de Gel QIAquick, de conformidad con

las instrucciones del fabricante. Se usó una columna (con un máximo de 400 mg de gel de agarosa por columna) y el ADN unido se eluyó con 50 µl de amortiguador EB. La concentración de ADN se determinó midiendo el OD_{260} (1 unidad de OD = 50 µg/ml). Una mezcla de ligación con un volumen final de 10 µl que contiene 100 ng de vector pPICZαA, linearizado con XhoI/EcoRI, 30 ng de VHH N-terminal, 30 ng de fragmento VHH C-terminal, 1 µl de amortiguador de ligación y 1 µl de ligada (3U), se preparó e incubó por 1 hora a temperatura ambiente. La transformación de *E. coli*, TG1, se realizó usando 2 µl de la mezcla de ligación. Las colonias se analizaron usando RCP como se describe en el documento WPA-0010, pero usando la combinación de cebador AOX1For/AOXIRev. El análisis de secuencia se realizó en clones positivos. La preparación del plásmido se realizó usando un Kit Miniprep de centrifugación Qiaprep (Qiagen), de conformidad con las instrucciones del fabricante y se describe anteriormente. El secuenciamiento se realizó en las instalaciones de secuencias VIB, Antwerp, Bélgica.

Transformación de P. pastoris

Véase Ejemplo 48.

El TNF30 se formateó a Nanobodies™ bivalentes. Se usó como espaciador entre los dos bloques de

construcción un enlazador GlySer de 30 AA. Para permitir las modificaciones específicas del sitio C-terminal, se introdujo una cisteína libre, ya sea como el último AA del Nanobody™, o con un espaciador adicional que consiste de GlyGlyGlyCys (SEC ID NO:471).

Ejemplo 56: Expresión y purificación de Nanobodies™ humanizados de vida media prolongada bivalentes

Producción en Pichia Pastoris

Véase Ejemplo 49

Purificación de Nanocuerpos bivalentes

El medio de cultivo se hizo libre de células vía centrifugación y filtración de 0.22 μm. El medio estéril se almacenó a 4°C hasta que es procesado adicionalmente. Los contaminantes de bajo peso molecular se redujeron vía ultra filtración en una membrana de ultrafiltración (UF) de 10kDa (HydroStart Sartcon Slice Casette, Sartorius) como sigue: se concentraron cuatro litros de medio a 0.5-1 litro, después se diluyeron con 5 litros de PBS y nuevamente se concentraron a 0.5 litros. Esta acción se realizó dos veces. Lo retenido de la UF se filtró a través de membranas de 47 mm de nylon, 0.45 μm (Alltech #2024).

En la siguiente etapa, se capturó el Nanobody™

bivalente a partir del medio concentrado vía purificación de afinidad de proteína A (usando MabSelectXtraTM, GE Healtcare). La columna [35x100 mm], se equilibró en PBS y después, la aplicación de muestra se lavó extensivamente con PBS. Se eluyó TNF56 con Glicina [100 mM, pH = 7.5].

Las fracciones eluidas de MabSelectXtraTM, fueron neutralizadas con Tris [1.5M, pH 8.8] y almacenadas a 4°C. Se concentró TNF56 y se purificó vía AEX (A= 10 mM de piperazina, pH 10.8 y B=1M de NaCl en 50 mM de Tris, pH 7.5), usando Source 30Q (GE Healthcare). En este extremo, las fracciones de Nanobody™ fueron diluidas con amortiguador A (10 mM de piperazina, pH 10.8), a una conductividad de 5 mS/cm y el pH se ajustó a 10.8. La columna [25x100 mm], se equilibró en amortiguador A antes de cargar la muestra en la columna. El TNF56 se eluyó con un gradiente de Volumen de Columna 5 (CV). El pH de las fracciones colectadas se ajustó a 7.8 usando 1M de Tris a pH = 7.8.

Pegilación de Nanobodies™ bivalentes expresados en *Pichia pastoris*

Reducción de cisteínas C-terminal

Se agregó Ditiotreitol (DTT, Aldrich Cat 15,046-0) a las fracciones neutralizadas para reducir los puentes de disulfuro potenciales que se forman entre las cisteínas

carboxi terminales de los Nanobodies™ (usualmente aproximadamente 20%). Una concentración final de 10 mM de DTT e incubación durante la noche a 4°C, se encuentra por ser óptima. La reducción se evaluó por cromatografía de exclusión de tamaño de analito (SEC). Por lo tanto, 25 µl del Nanobody™ reducido, se agregaron a 75 µl de D-PBS e inyectaron en una columna Sup75 10/300 GL, equilibrada en PBS de Dulbecco (D-PBS, GibcoTM REF 14190-094).

Se removió el Nanobody™ no reducido y el DTT por SEC preparativa en una columna de grado prep. Huload 20/60 Superdex75 equilibrada en D-PBS.

Se midió la concentración del Nanobody™ reducida, midiendo la absorbancia a 280 nm. Se usó un Espectrofotómetro UV/VIS de Doble Haz Uvikon 943 (método: véase SOP ABL-0038). Las absorciones se midieron en un explorador de longitud de onda de 245-330 nm. Se usaron dos células de precisión hechas de células Suprasil® Quartz (Hellma tipo No:104-QS; trayectoria ligera: 10 mm). Primero, la absorción del blanco se midió a 280 nm colocando dos células llenas por 900 µl de D-PBS. La muestra se diluyó (1/10) agregando 100 µl de la muestra a la primera célula. La absorción de la muestra se midió a 280 nm.

La concentración se calculó con la siguiente fórmula: $\frac{OD_{280}Muestra - OD280blanco}{}$ x 10

$$\varepsilon \times 1$$

Para TNF55: $\varepsilon = 1.85$

Para TNF56: $\varepsilon = 1.83$.

PEGilación

A Nanobody™ PEGilado, se agregó un exceso molar de 5X de solución PEG40 1 mM recientemente elaborada a la solución de Nanobody™ reducido. (MPEG2-MAL-40K de NEKTAR™.

Los terapéuticos de transformación (2D3YOTO1) Pm = 40,000 g/mol; MPEG2-MAL-60K de Terapéuticos de Transformación NEKTAR™ (2D3YOVO1) Pm = 60,000 g/mol).

La mezcla de Nanobody™-PEG, se incubó por 1 hora a temperatura ambiente (TA) con agitación suave y después se transfirió a 4°C. La PEGilación se evaluó vía SEC analítico. Por lo tanto, se agregaron 25 µl del Nanobody™ a 75 µl de D-PBS e inyectó en una columna Sup75HR 10/300, equilibrada en D-PBS. El Nanobody™ pegilado es eluido en el intervalo del volumen de exclusión de la columna (>75 kDa).

El Nanobody™ PEGilado y no PEGilado, se separó vía cromatografía de intercambio catiónico (CEX, usando Source30S, GE Healthcare; amortiguador A = 25 mM de ácido cítrico a pH = 4 y B = 1 M de NaCl en BPS). La muestra se diluyó a una conductividad de <5 mS/cm y el pH se ajustó a 4.0. La columna [25 x 100 mm], se equilibró y después la

aplicación de muestra se lavó extensivamente con amortiguador A. Se eluyó Nanobody™ pegilado con un gradiente de 3CV. El Nanobody™ colectado fue amortiguador intercambiado a D-PBS por SEC en una columna de grado prep Hiload 26/60 Superdex75, equilibrada en D-PBS.

Finalmente, el Nanobody™, se hizo libre de LPS vía el paso sobre una columna de intercambio aniónico (Source 30Q). La columna (10x100 mm), se sanitizó durante la noche en NaOH [1M] y posteriormente se equilibró en D-PBS libre de endotoxina.

Biotinilación

Para Biotinilar Nanobody™, se agregó un exceso molar de 5X de biotina (EZ-Link® Maleimida-PO2-Biotina, Pierce #21901) de una solución base de 10 mm, al Nanobody™ reducido (véase 5.5.1). La mezcla de Nanobody™ biotinilada, se incubó por 1 hora a TA con agitación suave y después se almacenó a 4°C.

La pureza del Nanobody™ biotinilado, se controló vía SEC analítico. Por lo tanto, se agregaron 25 µl de Nanobody™ biotinilado a 75 µl de D-PBS, y se inyectó en una columna Sup75HR 10/300, equilibrada en D-PBS. A partir del cromatograma obtenido, se podría concluir que la biotina del Nanobody™ no necesita purificación adicional: no se podría detectar dimerización de Nanobody™ vía una

oxidación de los sulfhidrilos libres. Un cambio de amortiguador a D-PBS, se hizo por un paso sobre una columna Sephadex G25 Fina (90 ml) de columna de desalación.

Finalmente, el Nanobody™ biotina se hizo libre de LPS por el paso sobre una columna de intercambio aniónico (Source30Q, GE Healtcare). La columna (1 x 10 cm), se sanitizó durante la noche en 1M de NaOH y después se equilibró en D-PBS.

Para determinar la pureza, las muestras de proteína se analizaron en gel de SDS-PAGE al 15%, como se describe en el ejemplo 8 y 49. Los resultados son presentados en la Figura 49 y 50.

Ejemplo 57: Caracterización de Nanobodies™ humanizados de vida media prolongada bivalentes.

Caracterización química

El TNF55 consiste de 260 aminoácidos. La proteína tiene un peso molecular de 27,100 Da. El pI es de 8.67. El coeficiente de extinción a 280 nm es 1.850.

El TNF56 consiste de 264 aminoácidos. La proteína tiene un peso molecular de 27,365 Da. El pI es de 8.67. El coeficiente de extinción a 280 nm es 1.830.

Espectrometría de masas

La masa teórica de TNF55 es 27,106 Da. La proteína TNF55-Biotina tiene 2 puentes S-S y una modificación de biotina, la cual debe resultar en una masa de 27,627 Da en ESI-EM. La masa que se determina experimentalmente para TNF55-biotina es 27,627 Da.

La masa teórica de TNF56 es 27,365 Da. La proteína TNF55-Biotina tiene 2 puentes S-S y una modificación de biotina, la cual debe resultar en una masa de 27,886 Da en ESI-EM. La masa que se determina experimentalmente para TNF55-biotina es 27,886 Da.

Secuenciamiento N-terminal

El secuenciamiento N-terminal de TNF56-PEG40, mostró que la secuencia de proteína para los primeros 7 aminoácidos es como sigue: EVQLVES. Esto es consistente con la secuencia de proteína teórica, la cual indica el procesamiento N-terminal apropiado.

Clasificación analítica

La clasificación analítica de TNF56-PEG40 es PBS, proporciona un pico simétrico. No se observaron contaminantes. El tiempo de retención observado es 8.5 ml en Superdex HR 75 y 10.32 ml en Superdex HR 200. Se muestra un perfil representativo en las Figuras 51 y 52.

Ejemplo 58: Funcionalidad en el ensayo a base de célula

La potencia para neutralizar la actividad citotóxica de TNFα, fue analizada en un ensayo a base de células. La potencia se examinó a diferentes concentraciones de Nanobody™, así como también de Enbrel, Humira y Remicade comercialmente disponibles, en una base molar. A superior EC50 observado, inferior la actividad del compuesto para neutralizar TNFα.

Los resultados son resumidos en la Tabla 45 y las Figuras 53 y 54.

Los datos muestran un incremento en potencia para los Nanobodies™ bivalentes, cuando se comparan con el TNF1 del Nanobodi™ monovalente. La potencia de los derivados de TNF55 es similar a la de los derivados de TNF56, la cual está en el intervalo de Enbrel y 10 veces mejor que el Humira y Remicade.

Ejemplo 59: Análisis de inmunogenicidad y farmacocinética de Nanocuerpos humanizados de vida media prolongada bivalentes en ratones

Véase ejemplo 54.

Los experimentos se realizaron para examinar la vida media de Nanobodies™ pegilados en ratones. La vida media de TNF56-PEG40 bivalente, se comparó con la vida media de TNF56-PEG60. Ambos Nanobodies™ tienen vida media

comparable de ~ 2 días. Los resultados están presentados en la Figura 55.

Además, la vida media del 3E-3E bivalente pegilado, se exploró. La vida media del 3E-3E-PEG20, se comparó con la vida media de 3E-3E-PEG40 después de la administración intravenosa de 100 μg de los Nanobodies™. 3E-3E-PEG20, tienen una vida media de 17 horas, mientras el 3E-3E-PEG40, tiene una vida media de 2.1 días, comparable con la vida media de 3E-3E-MSA21. Los resultados están presentados en la Figura 56.

Las muestras de suero fueron diluidas 100 veces y analizadas para determinar la presencia de anticuerpos anti-TNF56-PEG40 o anti-TNF56-PEG60. La carencia de inmunogenicidad se demostró para ambas moléculas. Los datos se presentan en la Figura 57.

Ejemplo 60: Eficacia de Nanocuerpo anti-TNF-α TNF60 (TNF60) en prevención de poliartritis crónica

Las líneas de ratón transgénico que portan y expresan el gen del factor necrosis del tumor humano 3'-modificado (hTNF-alfa; caquectina), se usaron como un modelo para estudiar la eficacia de TNF60 (TNF60) en la prevención del desarrollo de artritis (EMBO J. 10, 4025-4031). Estos ratones han sido mostrados por desarrollar poliartritis crónica con 100% de incidencia en cuatro a

siete semanas de edad.

A partir de la tercera semana de edad, camadas de ratones transgénicos se dividieron en grupos de ocho animales. Antes de inicializar el estudio, el peso corporal promedio se calculó para cada grupo. A partir de entonces, durante el estudio completo los pesos del animal se registraron una vez por semana para cada grupo.

Para probar la eficacia de TNF60 en la prevención de poliartritis crónica, se dieron inyecciones intraperitoneales dos veces por semana a cada animal de un grupo particular de conformidad con el siguiente esquema:

-Grupo 1 (control negativo): salina amortiguada de fosfato (PBS) (amortiguador de formulación).

-Grupo 2 (Tratamiento de Nanocuerpo): TNF60 a una dosis final de 30 mg/kg.

-Grupo 3 (Tratamiento de Nanocuerpo): TNF60 a una dosis final de 10 mg/kg.

-Grupo 4 (Tratamiento de Nanocuerpo): TNF60 a una dosis final de 3 mg/kg.

-Grupo 5 (1er. control positivo): Enbrel a una dosis final de 30 mg/kg.

-Grupo 6 (1er. control positivo): Enbrel a una dosis final de 10 mg/kg.

-Grupo 7 (2do. control positivo): Remicade a una dosis final de 30 mg/kg.

-Grupo 8 (2do. control positivo): Remicade a una dosis final de 10 mg/kg.

-Grupo 9 (2do. control positivo): Remicade a una dosis final de 3 mg/kg.

Para cada grupo, los datos de inyección y volúmenes fueron indicados.

Las inyecciones continuaron por varias semanas. Durante este periodo, los registros clínicos fueron registrados observando cambios macroscópicos en la morfología de articulación para cada animal.

A 10 semanas de edad, todos los ratones fueron sacrificados y se colectaron las articulaciones y suero. El suero se almacenó a -70°C y las articulaciones de la tobillo fueron conservadas en formalina.

Para los grupos seleccionados, las articulaciones de tobillo fueron embebidas en parafina y seccionadas. Las secciones de articulación del tobillo fueron subsecuentemente usadas para evaluación histopatológica del progreso de la enfermedad.

Los resultados están representados en la Figura 58.

Ejemplo 61: Eficacia de Nanocuerpo TNF60 anti-TNF-α (TNF60) en tratamiento terapéutico de poliartritis crónica

Las líneas de ratón transgénico que portan y

expresan el gen del factor necrosis del tumor humano 3'-modificado (hTNF-alfa; caquectina), se usaron como un modelo para estudiar la eficacia de TNF60 (TNF60) en el tratamiento terapéutico de artritis (EMBO J. 10, 4025-4031). Estos ratones han sido mostrados por desarrollar poliartritis crónica con 100% de incidencia en cuatro a siete semanas de edad.

A partir de la sexta semana de edad, camadas de ratones transgénicos se dividieron en grupos de ocho animales. Antes de inicializar el estudio, el peso corporal promedio se calculó para cada grupo. A partir de entonces, durante el estudio completo los pesos del animal se registraron una vez por semana para cada grupo.

Para probar la eficacia de TNF60 en el tratamiento terapéutico de poliartritis crónica, se dieron inyecciones intraperitoneales dos veces por semana a cada animal de un grupo particular de conformidad con el siguiente esquema:

-Grupo 1 (control negativo): salina amortiguada de fosfato (PBS) (amortiguador de formulación).

-Grupo 2 (Tratamiento de Nanocuerpo): TNF60 a una dosis final de 30 mg/kg.

-Grupo 3 (Tratamiento de Nanocuerpo): TNF60 a una dosis final de 10 mg/kg.

-Grupo 4 (1er. control positivo): Enbrel a una

dosis final de 30 mg/kg.

-Grupo 5 (2do. control positivo): Remicade a una dosis final de 30 mg/kg.

Para cada grupo, los datos de inyección y volúmenes fueron indicados.

Las inyecciones continuaron por varias semanas. Durante este periodo, los registros clínicos fueron registrados observando cambios macroscópicos en la morfología de articulación para cada animal.

A 13 semanas de edad, todos los ratones fueron sacrificados y se colectaron las articulaciones y suero. El suero se almacenó a -70°C y las articulaciones de la tobillo fueron conservadas en formalina.

Para los grupos seleccionados, las articulaciones de tobillo fueron embebidas en parafina y seccionadas. Las secciones de articulación del tobillo fueron subsecuentemente usadas para evaluación histopatológica del progreso de la enfermedad.

Los resultados están representados en la Figura 59.

Ejemplo 62: Efecto de formateado en la eficacia de un Nanocuerpo anti-TNFα en la prevención de poliartritis crónica

Las líneas de ratón transgénico que portan y

expresan el gen del factor necrosis del tumor humano 3'-modificado (hTNF-alfa; caquectina), se usaron como un modelo para estudiar la eficacia de un Nanocuerpo anti-TNFα formateado en diferentes formas, en la prevención de poliartritis crónica (EMBO J. 10, 4025-4031). Estos ratones han sido mostrados por desarrollar poliartritis crónica con 100% de incidencia en cuatro a siete semanas de edad.

A partir de la tercera semana de edad, camadas de ratones transgénicos se dividieron en grupos de ocho animales. Antes de inicializar el estudio, el peso corporal promedio se calculó para cada grupo. A partir de entonces, durante el estudio completo los pesos del animal se registraron una vez por semana para cada grupo.

Para probar la eficacia del Nanocuerpo anti-TNFα en diferentes formatos para la prevención de poliartritis crónica, se dieron inyecciones intraperitoneales dos veces por semana a cada animal de un grupo particular de conformidad con el siguiente esquema:

-Grupo 1 (control negativo): salina amortiguada de fosfato (PBS) (amortiguador de formulación).

-Grupo 2 (formato de Nanocuerpo 1): TNF60 a una dosis final de 10 mg/kg.

-Grupo 3 (formato de Nanocuerpo 1): TNF60 a una dosis final de 2.5 mg/kg.

-Grupo 4 (formato de Nanocuerpo 1): TNF60 a una

dosis final de 1 mg/kg.

-Grupo 5 (formato de Nanocuerpo 2): TNF-56-PEG40 a una dosis final de 10 mg/kg.

-Grupo 6 (formato de Nanocuerpo 2): TNF-56-PEG40 a una dosis final de 1.8 mg/kg.

-Grupo 7 (formato de Nanocuerpo 2): TNF-56-PEG40 a una dosis final de 0.7 mg/kg.

-Grupo 8 (formato de Nanocuerpo 3): TNF-56-biotina a una dosis final de 1.8 mg/kg.

-Grupo 9 (formato de Nanocuerpo 4): TNF-30 a una dosis final de 1 mg/kg.

-Grupo 10 (formato de Nanocuerpo 5): TNF1 a una dosis final de 1 mg/kg

-Grupo 11 (1er. Control positivo): Enbrel a una dosis final de 10 mg/kg

-Grupo 12 (2do. Control positivo): Remicade a una dosis final de 10 mg/kg.

Para cada grupo, los datos de inyección y volúmenes fueron indicados.

Las inyecciones continuaron por varias semanas. Durante este periodo, los registros clínicos fueron registrados observando cambios macroscópicos en la morfología de articulación para cada animal.

A 10 semanas de edad, todos los ratones fueron sacrificados y se colectaron las articulaciones y suero. El

suero se almacenó a -70°C y las articulaciones de la tobillo fueron conservadas en formalina.

Para los grupos seleccionados, las articulaciones de tobillo fueron embebidas en parafina y seccionadas. Las secciones de articulación del tobillo fueron subsecuentemente usadas para evaluación histopatológica del progreso de la enfermedad.

Los resultados están representados en la Figura 60.

Ejemplo 63: Estudio de farmacocinética de Nanocuerpos TNF60 anti-TNF-α (TNF60) y TNF56-PEG40 en monos Rhesus

Monos Rhesus reproducidos cautivos (*Macaca mulatta*), se usaron para determinar el perfil de farmacocinética de TNF60 y TNF56-PEG40.

Se usaron dieciséis animales en este estudio (ocho hembras y ocho machos) y se dividieron en cuatro grupos (dos machos y dos hembras por grupo). Todos los animales pesaron aproximadamente 5 kg y están libres de enfermedad por al menos, seis semanas antes del uso. El Sniff® Pri vegetarisch V3994 sirve como alimento. Sesenta g/kg de b.w. son ofreció a cada mono. El residuo es removido. A intervalos regulares (al menos dos veces por año), el alimento se analizó basado en EPA/USA por contaminantes por LUFA-ITL. El agua de llave es ofrecida a

albedrío. Los animales en cada grupo de tratamiento son alojados en un bloque de varias jaulas adyacentes dentro de la unidad del mono. Los monos son mantenidos individualmente en jaulas de acero V_2A con un tamaño de 90 cm x 82 cm x 96 cm. La temperatura ambiente es mantenida a 23°C $\pm$ 3°C (intervalo máximo) y la humedad relativa a 60% $\pm$ 20% (intervalo máximo). Las desviaciones a partir del intervalo máximo causadas por ejemplo, durante el procedimiento de limpieza, son considerados en SOPs. Los cuartos son iluminados y oscurecidos por periodos de 12 horas cada uno.

Los dos grupos son infundidos con TNF60 y dos grupos son infundidos con TNF56-PEG40. Las infusiones intravenosas de TNF60 y TNF56-PEG40 (disuelto en PBS) en la vena cefálica del brazo derecho e izquierdo, usando catéteres permanentes y una bomba de infusión TSE (véase posteriormente), se da una dosis fija de 2 mg/kg.

Se realizaron cuatro administraciones únicas, separadas por un periodo de lavado de al menos, catorce días. Después de la última administración, el periodo de seguimiento es al menos, de ocho semanas. Dos de los cuatro grupos son tratados con TNF60 ó TNF56-PEG40 en combinación con metotrexato (MTX) (disuelto en PBS). El grupo 2 es tratado con TNF60 y MTX; el grupo 4 es tratado con TNF56-PEG40 y MTX. El MTX es dosificado semanalmente

intramuscularmente a 0.2 mg/kg. En los días de administración, el MTX es dado aproximadamente 30 minutos antes de la administración. La dosificación inicia en la primera administración de Nanocuerpo y continuará a través de las ocho semanas de lavado después de la cuarta dosis. Existen catorce administraciones MTX únicas, separadas por un periodo de lavado de al menos, una semana, iniciando con la primera administración de punto de prueba.

Ejemplo 64: Estudios de fibroblastos derivados del sinovio

En este estudio, se valoró la capacidad de los biológicos anti-TNF, ALX0071 y Etanecerpt, para atenuar la producción de IL-6 inducida por TNF-α por fibroblastos derivados del sinovio RA.

Aislamiento de fibroblastos sinoviales

Tejidos de articulación sinovial a partir de pacientes RA consentidos, se almacenó en medio a base de DMEM, con antibióticos a 4°C por hasta 96 horas después de la cirugía de reemplazo de articulación. Las células sinoviales fueron aisladas a partir del sinovio disectado por digestión de colagenasa a 37°C por 2 horas. La suspensión celular resultante entonces se lavó por una serie de etapas de centrifugación y resuspensión y las células resultantes entonces se cultivaron a 37°C en medio

de cultivo a base de DMEM suplementado con FCS al 10% (v/v). Los fibroblastos resultantes se usaron para el siguiente experimento ya sea en el segundo o tercer paso. Las células de cuatro donadores, fueron usadas en experimentos individuales. Los fibroblastos fueron sembrados en placas de poliestireno de fondo plano de 96 cavidades a 1.5×10^4 células en un volumen final de 250 µl de medio de cultivo a base de DMEM suplementado con FCS al 10% (v/v) por cavidad y cultivadas durante la noche.

Estimulación del fibroblasto sinovial

Las células son entonces incubadas por 72 horas en medio de cultivo a base de DMEM, suplementadas con TNFα a 50 ng por ml (3 nM (R&D Systems 210-TA/CF) solo o en la presencia de dosis incrementadas de ALX0071 (0.575 hasta 1920 ng por ml; 0.015 hasta 50 nM) o Etanercept (Wyeth Labs; 3.75 a 11250 ng por ml; 0.025 hasta 75 nm). El volumen final en cada cavidad fue de 250 µl y cada valoración se realizó por triplicado. Después de 72 horas, el medio sobrenadante se removió y almacenó a -40°C antes del análisis por IL-6 ELISA (R&D Systems). La inhibición de producción de IL-6 inducida por TNF-α, se determinó y los valores IC_{50} fueron calculados para tanto ALX0071 como Etanercept.

Sumario de resultados

La producción de IL-6 inducida por TNFα es dependientemente reducida de dosis tanto de ALX10071 como Etanercept, por fibroblastos derivados del sinovio RA de cuatro donadores. Existe una potencia similar entre los dos reactivos bajo estas condiciones de ensayo.

Ejemplo 65: Estudios de bolsas de Aire Murina

En este estudio, se valoró la capacidad de los biológicos anti-TNF, ALX0071 y Etancerpt, para atenuar la infiltración celular inducida por TNFα en una bolsa de aire de murina.

Creación de Bolsas de Aire

Las bolsas de aire se formaron por inyección sub-cutánea (s.c.) de 2.5 ml de aire estéril en la superficie dorsal de ratones machos anestesiados C57B1/6/J (25-30 g, Harlan). La bolsa se reinfló inyectando 2.5 ml de aire estéril 3 días después.

Estimulación de TNFα

Seis días después de la creación inicial de la bolsa de aire, los animales fueron anestesiados y la bolsa inyectada con 1 ml de vehículo de carboximetilcelulosa al 0.5% (CMC), que contiene 0.1 µg de TNFα recombinante humana

(R&D Systems, 210-TA-050/CF). En los otros tres grupos de animales, se inyectó ALX0071 (0.0625, 0.125 y 0.25 mg/kg) s.c. 19 horas antes de la inyección de TNFα. Se inyectó un segundo grupo de tres animales (s.c.) con Etanercept (Wyeth Labs, 0.125, 0.25 y 0.5 mg/kg), inmediatamente antes de la inyección de TNFα.

24 Horas después de la inyección por TNFα, los ratones fueron elegidos con una concentración elevada de CO_2. Las bolsas fueron lavadas con 2 ml de PBS estéril libre de endotoxina enfriado en huelo que contiene 5 IU/ml de heparina. Los volúmenes fueron registrados y se separaron 0.5 ml de alícuotas para conteo de la población de células blancas de la sangre totales (WBC) en un contador celular Sysmex XT-Vet. El error estándar y la media de los conteos WBC totales medios (SEM) para cada grupo, fueron calculados por ml de fluido de lavado retirado. Los análisis estadísticos fueron por ANOVA con datos no transformados y posteriores a la prueba Kruskal-Wallis.

Sumario de resultados

Tanto ALX0071 como Etanercept, atenuaron la infiltración de WBC inducida por TNFα en las bolsas de aire (Tabla). Mientras esta atenuación alcanza significancia estadística en tanto 0.125 ($P<0.01$) como 0.25 mg/kg

(P<0.05). Los grupos de dosis de ALZ0071, no se observó significancia estadística con cualquiera de los grupos de dosis de Etanercept.

Tabla 8

Nombre	SEC ID NO	Secuencia
PMP1C2(TNF1)	52	QVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTA LYYCARSPSGFNRGQGTQVTVSS
PMP1G11	53	QVQLQESGGGMVQPGGSLRLSCAASGFDFGVSWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKTTLYLQMNSLKPEDTA LYYCARSPSGSFRGQGTQVTVSS
PMP1H6	54	EVQLVESGGGLVQPGGSLRLSCATSGFDFSVSWMYWVRQAPGKGLE WVSEINTNGLITKYVDSVKGRFTISRDNAKNTLYLQMDSLIPEDTA LYYCARSPSGSFRGQGTQVTVSS
PMP1G5(TNF2)	55	QVQLVESGGGLVQAGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKEREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLLMNSLK PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSS
PMP1H2	56	QVKLEESGGGLVQPGDSLRLSCAASGRTFSDYSGYTYTVGWFRQAP GKEREFVARIYWSSGNTYYADSVKGRFTISRDIAKNTVDLLMNNLE PEDTAVYYCAARDGIPTSRSVESYNYWGQGTQVTVSS
PMP3G2	57	AVQLVESGGGLVQPGDSLRLSCAASGRTFSDYSGYTYTVGWFRQAP GKEREFVARIYWSSGNTYYADSVKGRFTISRDIAKNTVDLLMNNLE PEDTAVYYCAARDGIPTSRSVESYNYWGQGTQVTVSS
PMP1D2	58	AVQLVDSGGGLVQAGGSLRLSCAASGRTFSAHSVYTMGWFRQAPGK EREFVARIYWSSANTYYADSVKGRFTISRDNAKNTVDLLMNCLKPE DTAVYYCAARDGIPTSRSVEAYNYWGQGTQVTVSS
PMP3D10	59	QVQLVESGGGLVQAGGSLSLSCAASGRSFTGYYMGWFRQAPGKERQ LLASISWRGDNTYYKESVKGRFTISRDDAKNTIYLQMNSLKPEDTA VYYCAASILPLSDDPGWNTNWGQGTQVTVSS
PMP5F10(TNF3)	60	EVQLVESGGGLVQAGGSLSLSCSASGRSLSNYYMGWFRQAPGKERE LLGNISWRGYNIYYKDSVKGRFTISRDDAKNTIYLQMNRLKPEDTA VYYCAASILPLSDDPGWNTYWGQGTQVTVSS
PMP6A8(ALB2)	61	AVQLVESGGGLVQGGGSLRLACAASERIFDLNLMGWYRQGPGNERE LVATCITVG.DSTNYADSVKGRFTISMDYTKQTVYLHMNSLRPEDT GLYYCKIRRTWHSELWGQGTQVTVSS
PMP6B4	62	EVQLVESGGGLVQEGGSLRLACAASERIWDINLLGWYRQGPGNERE LVATITVG.DSTSYADSVKGRFTISRDYDKNTLYLQMNSLRPEDTG LYYCKIRRTWHSELWGQGTQVTVSS
PMP6A6(ALB1)	63	AVQLVESGGGLVQPGNSLRLSCAASGFTFRSFGMSWVRQAPGKEPE WVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTA VYYCTIGGSLSRSSQGTQVTVSS
PMP6C1	64	AVQLVDSGGGLVQPGGSLRLSCAASGFSFGSFGMSWVRQYPGKEPE WVSSINGRGDDTRYADSVKGRFSISRDNAKNTLYLQMNSLKPEDTA EYYCTIGRSVSRSRTQGTQVTVSS
PMP6G8	65	AVQLVESGGGLVQPGGSLRLTCTASGFTFRSFGMSWVRQAPGKDQE WVSAISADSSTKNYADSVKGRFTISRDNAKKMLYLEMNSLKPEDTA VYYCVIGRGSPSSPGTQVTVSS

PMP6A5	66	QVQLAESGGGLVQPGGSLRLTCTASGFTFGSFGMSWVRQAPGEGLE WVSAISADSSDKRYADSVKGRFTISRDNAKKMLYLEMNSLKSEDTA VYYCVIGRGSPASQGTQVTVSS
PMP6G7	67	QVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMYWVRVAPGKGLE RISRDISTGGGYSYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDT ALYYCAKDREAQVDTLDFDYRGQGTQVTVSS
NC55TNF_S1C4	105	EVQLVESGGGLVQAGDSLRLSCAASQIIFGSHVAAWFRQAPGRERE FVAEIRPSGDFGPEGEFEHVTASLKGRFTIAKNSVDNTVYLQMNSL KPEDTAVYYCAAAPYRGGRDYRWEYEYEYWGQGTQVTV
NC55TNF_S1C3	106	EVQLVESGGGLVQPGGSLRLSCKNAGSTSNAYATGWFRRAPGKERE FVAGIQWSGGDAFYRNSVKGRFRITRDPDNTVYLQMNDLKPEDTAI YYCAQKLSPYYNDFDSSNYEYWGQGTQVTV
NC55TNF_S2C1	107	EVQLVESGGDLVQPGGSLRLSCAVSGQLFSTNDVGWYRRAPGKQRE LVATITDDGTTDYGDDVKGRFVISREGEMVYLEMNSLKPEDTAVYY CNINRLRSTWGIRYDVWGQGTQVTVSS
NC55TNF_S2C5	108	EVQLVESGGGLVQPGGSLRLSCVVSGFTFSTTSMTWVRQAPGKFEE WVSFINSDGSSTTYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTA MYYCGRRGYGRDRSKGIQVTVAS
NC55TNF_S3C7	109	EVQLVESGGGTVQAGDSLRLSCAASGRSFSSVAMGWFRQAPGKQRE FLAGVGYDGSSIRYAESVKGRFTIARGNRESTVFLQMENLKPEDTA VYFCTAEPIGAYEGLWTYWGQGTQVTVSS
NC55TNF_S3C1	110	XXXXVESGGGLMQPGGSLKLSCAASGFMFSDSAMGWFRQAPGKERE FVATISWNGGSSSYADFVKGRFTISRDNAKNTVYLQMNGLTPQDTA IYYCAGSYSNGNPHRFSQYQYWGQGTQVTVSS
NC55TNF_BMP1B2	111	EVQLVESGGGLVQAGGSLRLSCAASGRTFGTYAMGWFRQAPGKERE FVAAISWGGGSIVYAESAKGRFTISRDNAKXTMYLQMDSLKPEDTA VYYCAAANNIATLRQGSWGQGTQVTVSS
NC55TNF_BMP1D2	112	EVQLVESGGELVQAGGSLKLSCTASGRNFVTYAMSWFRRAPGKERE FVASISWSGDTTYYSNSVKGRFTVSRDNGKNTAYLRMNSLKPEDTA DYYCAVVQVIDPSWSGVNLDDYDYLGSGTQVTVSS
NC55TNF_BMP1E2	113	EVQLVESGGRLVQPGGSLRLSCKNAGSTSNAYATGWFRRAPGKERE FVAGIQWSGGDAFYRNSVKGRFRITRDPDNTVYLQMNDLKPEDTAI YYCAQKLSPYYNDFDSSNYEYWGQGTQVTVSS
NC55TNF_BMP1G2	114	EVQLVESGGGLVQPGGSLRLSCAASATISSIVMLGWYRQAPGKQRE WVASITIGSRTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAV YFCNAVPPRDDYWGQGTQVTVSS
NC55TNF_BMP2A2	115	EVQLVESGGGLVQAGGSLRLSCAASGQTSSSYDMGWFRQAPGEGRE FVARISGSDGSTYYSDRAKDRFTISRDNTKNMVYLQMDRLKPDDTA VYYCRVPRYENQWSSYDYWGQGTQVTVSS
NC55TNF_BMP2C2	116	EVQLVESGGGLVQPGGSLRLSCAASGSTFSTYDMSWVRQAPGKGLE WVSGIDSGGGSPMYVDSVKGRFTVSRDNAKNTLYLQMNSLKPEDTA VYYCAKFSTGADGGSWYWSYGMDSWGKGTQVTVSS
NC55TNF_BMP2F2	117	EVQLVESGGGLVQAGDSLRLSCEASERSSNRYNMAWFRQAPGKERE FLARVDVSGGNTLYGDSVKDRFTVSRINGKNAMYLQMNNLKPEDTA IYYCAAGGWGTTQYDYDYWGQGTQVTVSS
NC55TNF_NC10	118	EVQLVESGGGLVQPGGSLRLSCVCVSSGCTFSAYSMTWVRQAPGKA EEWVSFINSDGSSTTYADSVNGRFKISRDNAKKTLYLQMNSLGPED TAMYYCQRRGYALDRGQGTQVTVSS
NC55TNF_NC11	119	EVQLVESGGGLVQAGDSLTLSCASSGRGFYKNAMGWFRQPPGKERE FVASIKWNGNNTYYADSVRGRFTISRGNAKNTENTVSLQMNSLKPE DTADYYCAADSSHYSYVYSKAYEYDYWGQGTQVTVSS
NC55TNF_NC1	120	EVQLVESGGGLVQPGGSLRLSCVFSGFAFSASSMAWVRQAPGKYEE WVSFINSDGSSTTYADSVQGRFTISRDNAKNTLYLQMNSLKSEDTA MYYCGRRGYGRDRSQGIQVTVSS
NC55TNF_NC2	121	EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAMGWFRQAPGKERE FVAAISWSGTITNYADSVKGRFTISRDNGKNTVHLQMNSLKPEDTA VYHCAVVQPYSGGDYYTGVEEYDYWGXGTQVTVSS

1190

NC55TNF_NC3	122	EVQLVESGGGLVQPGGSLRLSCVVSGFTFSATSMTWVRQAPGKAEE WVSFINSDGSSTTYADSVKGRFTISRDNAKNTLYLQMDDLQSEDTA MYYCGRRGYGRDRSRGIQVTVSS
NC55TNF_NC5	123	EVQLVESGGGLVQAGGSLRLSCAASGGAFSNYDVGWFRQAPGEGRE IVARISGSGDSTYSSNRAKGRFTISRDNAKNTVYLQMNSLKREDTA VYYCRAARYNGTWSSNDYWGQGTQVTVSS
NC55TNF_NC6	124	EVQLVESGGGLVQPGGSLRLSCECVSSGCTFSAYSMTWVRQAPGKA EEFVSFINSDGSSTTYANSVNGRFKISRDNAKKTLYLQMNSLGPED TAMYYCQRRGYALDRGQGTQVTVSS
NC55TNF_NC7	125	QVQLVESGGGLVQAGGSLRLSCTASGQTSSTADMGWFRQPPGKGRE FVARISGIDGTTYYDEPVKGRFTISRDKAQNTVYLQMDSLKPEDTA VYYCRSPRYADQWSAYDYWGQGTQVTVSS
NC55TNF_NC8	126	EVQLVESGGGLVQPGGSLRLSCVVSGFTFSTTSMTWVRQAPGKFEE WVSFINSDGSSTTYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTA MYYCGRRGYGRDRSKGIQVTVSS
NC55TNF_S2C2	127	EVQLVESGGGLVQPGGSLRLSCVASASGVKVNDMGWYRQAPGKERE LVATITDDGRTNYEDFAKGRFTISRDNAKNTVYLQMNSLLPEDTAV YYCNARTYWAHLPTYWGQGTQVTVSS
NC55TNF_S1C6	128	EVQLVESGGGLVQAGGSLRLSCAASGRSFGSVAMGWFRQAPGKERE FVAAIGYDGNSIRYGDSVKGRFTISRDNIKNTMYLEMENLNADDTA RYLCAAEPLARYEGLWTYWGQGTQVTVSS
NC55TNF_S3C2	129	EVQLVESGGGLVQAGASLRLSCTTSTRTNDRFNMAWFRQAPGKDRE FVSRIDVAGYNTAYGDFVKGRFTVSRDSAENTVVLQMNSLRPEDTG VYYCAAGGWGISQSDYDLWGQGTQVTVSS

Tabla 9

nanocuerpo	Clase	Koff estimado (1/s)
PMP5 F10	III	2,63E-04
PMP1 G5	II	3,59E-04
PMP1 C2	I	4,39E-04
PMP1 G11	I	1,15E-03
PMP1 H6	I	2,14E-03
PMP1 H2	II	3,65E-03
PMP3 G2	II	1,09E-02

1191

Tabla 10

Nanocuerpo	Secuencia de línea germinal	diferencias #AA/ #AA toal	%AA de identidad
ALB1	DP51/DP53	13/87	85.1
ALB2	DP54	26/87	70.2
TNF1	DP51/DP53	6/87	93.2
TNF2	DP54	16/87	81.7
TNF3	DP29	18/87	79.4

Tabla 11

Nanocuerpo	Tiempo de inducción	Rendimiento (mg/l)
ALB1	corto/37°C	18
ALB2	corto/37°C	4
TNF1	corto/37°C	8.3
TNF2	corto/37°C	5
TNF3	corto/37°C	0.8

Tabla 12

	50% enlace (ng/ml)	
ID	TNFα humano	TNFα Rhesus
TNF1	12	12
TNF2	20	>3000
TNF3	18	16

1192

Tabla 13

.	50% de inhibición (ng/ml)	
ID	TNFα humano	TNFα Rhesus
TNF1	530	220
TNF2	3500	>5000
TNF3	100	100

Tabla 14

TNFα humano	Kd (1/s)
TNF1	1,05E-03
TNF2	1,33E-03
TNF3	3,02E-04

Tabla 15

		Albúmina humana	Albúmina Rhesus	Albúmina de ratón
ALB1	KD (nM)	0,57	0,52	6,5
	ka (1/Ms)	1,11E+06	1,05E+06	1,11E+06
	kd (1/s)	6,30E-04	5,46E-04	7,25E-03
ALB2	KD (nM)	0,092	0,036	15,7
	ka (1/Ms)	8,15E+05	1,94E+06	1,95E+05
	kd (1/s)	7,52E-05	7,12E-05	3,07E-03

Tabla 16:

Ensayo: *L929s + Act D (5000c/w)*

TNF: TNFα humano @ *0.5ng/ml*

		EC_{50} en nM			potencia relativa	
Nanocuerpo		media	desv. est.	#	media	desv.est.
TNF1	1C2	0.707	0.265	14	0.015	0.007
TNF2	1G5	1.412	0.622	14	0.007	0.002
TNF3	5F10	0.224	0.133	14	0.048	0.019
Enbrel		0.009	0.005	45	1.002	0.011
Humira		0.079	0.043	39	0.097	0.069
Remicade		0.083	0.037	45	0.103	0.058

ensayo: *L929s + Act D (5000c/w)*

TNF: TNFα *rhesus* @ *0.5ng/ml*

		EC_{50} en nM			potencia relativa	
Nanocuerpo		media	desv.est.	#	media	desv.est.
TNF1	1C2	0.693	0.305	9	0.015	0.009
TNF2	1G5	>50		9		
TNF3	5F10	0.602	0.283	9	0.017	0.010
Enbrel		0.009	0.003	7	1	0.000
Humira		0.071	0.025	8	0.103	0.059
Remicade		>6.7		7		

Tabla 17

%	Sin tratar	TA	37°C	50°C	60°C	70°C	80°C	90°C
TNF1	100	98	98	98	98	95	92	90
TNF2	100	99	100	99	97	96	63	50
TNF3	100	96	97	98	96	94	75	70
ALB1	100	101	102	101	100	64	94	90
ALB2	100	100	102	100	100	28	8	17

1194

Tabla 18

nanocuerpo	temp en °C	EC_{50} en nM	#	potencia relativa
TNF1	control	0,916	1	0,013
92#2302nr1.TNF1	TA	0,873	1	0,014
	37	0,901	1	0,013
	50	0,908	1	0,013
	60	0,891	1	0,013
	70	1,218	1	0,010
	80	2,655	1	0,004
	90	5,797	1	0,002
TNF2	control	2,500	1	0,005
92#2302nr2.TNF2	TA	2,165	1	0,005
	37	2,212	1	0,005
	50	2,241	1	0,005
	60	1,782	1	0,007
	70	2,487	1	0,005
	80	2,818	1	0,004
	90	6,135	1	0,002
TNF3	control	0,278	1	0,043
92#2302nr3.TNF3	TA	0,289	1	0,041
	37	0,295	1	0,040
	50	0,290	1	0,041
	60	0,281	1	0,042
	70	0,293	1	0,040
	80	0,576	1	0,021
	90	0,861	1	0,014

1195

Tabla 19

Nombre	SEC ID NO	Secuencia
GS9	69	GGGGSGGGS
GS30	69	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGS
TNF1-GS9-TNF1(TNF4)	70	QVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTALYYCARSPSGFNRGQGTQVTVSSGGGGSGGGSQVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTALYYCARSPSGFNRGQGTQVTVSS
TNF2-GS9-TNF2(TNF5)	71	QVQLVESGGGLVQAGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAPGKEREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLLMNSLKPEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSSGGGGSGGGSQVQLVESGGGLVQAGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAPGKEREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLLMNSLKPEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSS
TNF3-GS9-TNF3(TNF6)	72	EVQLVESGGGLVQAGGSLSLSCSASGRSLSNYYMGWFRQAPGKEREFLLGNISWRGYNIYYKDSVKGRFTISRDDAKNTIYLQMNRLKPEDTAVYYCAASILPLSDDPGWNTYWGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQAGGSLSLSCSASGRSLSNYYMGWFRQAPGKEREFLLGNISWRGYNIYYKDSVKGRFTISRDDAKNTIYLQMNRLKPEDTAVYYCAASILPLSDDPGWNTYWGQGTQVTVSS
TNF1-GS30-TNF1(TNF7)	73	QVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTALYYCARSPSGFNRGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSQVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTALYYCARSPSGFNRGQGTQVTVSS
TNF2-GS30-TNF2(TNF8)	74	QVQLVESGGGLVQAGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAPGKEREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLLMNSLKPEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSQVQLVESGGGLVQAGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAPGKEREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLLMNSLKPEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSS
TNF3-GS30-TNF3(TNF9)	75	EVQLVESGGGLVQAGGSLSLSCSASGRSLSNYYMGWFRQAPGKEREFLLGNISWRGYNIYYKDSVKGRFTISRDDAKNTIYLQMNRLKPEDTAVYYCAASILPLSDDPGWNTYWGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLSLSCSASGRSLSNYYMGWFRQAPGKEREFLLGNISWRGYNIYYKDSVKGRFTISRDDAKNTIYLQMNRLKPEDTAVYYCAASILPLSDDPGWNTYWGQGTQVTVSS
TNF30-30GS-TNF30-C (TNF55)	419	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCARSPSGFNRGQGTLVTVSSggggsggggsggggsggggsggggsggggsEVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCARSPSGFNRGQGTLVTVSC
TNF30-30GS-TNF30-gggC (TNF56)	420	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCARSPSGFNRGQGTLVTVSSggggsggggsggggsggggsggggsggggsEVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCARSPSGFNRGQGTLVTVSSgggC

1196

Tabla 20

ID	Formato	Enlazador
TNF4	TNF1-TNF1	9 AA GlySer
TNF5	TNF2-TNF2	9 AA GlySer
TNF6	TNF3-TNF3	9 AA GlySer
TNF7	TNF1-TNF1	30 AA GlySer
TNF8	TNF2-TNF2	30 AA GlySer
TNF9	TNF3-TNF3	30 AA GlySer

Tabla 21

Nanobody™	Tiempo de inducción	Rendimiento (mg/L)
TNF4	ON/28°C	3.2
TNF5	corto/37°C	5.5
TNF6	corto/37°C	1.19
TNF7	ON/28°C	2.7
TNF8	corto/37°C	6.6
TNF9	ON/28°C	1.3

Tabla 22

	50% de inhibición (ng/ml)
ID	TNFα Humano
TNF4	13
TNF5	6.3
TNF6	30
TNF7	16
TNF8	23
TNF9	18

1197

Tabla 23

ensayo: *L929s + Act D (5000c/w)*

TNF: *TNFa humano @ 0.5ng/ml*

	EC_{50} en nM			potencia relativa	
Nanocuerpo	media	desv.est.	#	media	desv.est.
TNF4	0.236	0.049	4	0.033	0.012
TNF5	0.020	0.010	9	0.566	0.275
TNF6	0.078	0.047	8	0.179	0.168
TNF7	0.013	0.005	8	0.673	0.211
TNF8	0.007	0.002	2	1.240	0.137
TNF9	0.012	0.005	6	0.729	0.242
Enbrel	0.009	0.005	45	1.002	0.011
Humira	0.079	0.043	39	0.097	0.069
Remicade	0.083	0.037	45	0.103	0.058

ensayo: *L929s + Act D (5000c/w)*

TNF: *TNFa rhesus @ 0.5ng/ml*

	EC_{50} en nM			potencia relativa	
Nanocuerpo	Media	desv.est	#	media	desv.est
TNF4	0.141	0.025	4	0.065	0.015
TNF5	35.000	16.000	5	0.000	0.000
TNF6	0.398	0.074	6	0.024	0.003
TNF7	0.011	0.005	4	0.860	0.142
TNF8	1.026	0.444	2	0.010	0.001
TNF9	0.038	0.012	4	0.249	0.032
Enbrel	0.009	0.003	7	1.000	0.000
Humira	0.071	0.025	8	0.103	0.059
Remicade	>6.7		7		

Tabla 24

%	sin tratar	TA	37°C	50°C	60°C	70°C	80°C	90°C
TNF4	100	99	99	99	98	55	34	17
TNF5	100	99	101	99	98	92	26	22
TNF6	100	103	104	103	105	99	7	7
TNF7	100	100	100	98	96	66	33	40
TNF8	100	99	100	99	100	89	11	8
TNF9	100	101	101	101	101	99	17	18

M98

Tabla 25

Nombre	SEC ID NO	Secuencia
TNF13(TNF1 HUM1)	76	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTA VYYCARSPSGFNRGQGTQVTVSS
TNF14(TNF1 HUM2)	77	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTA LYYCARSPSGFNRGQGTLVTVSS
TNF15(TNF2 HUM1)	78	EVQLVESGGGLVQPGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKGREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLQMNSLK PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSS
TNF16(TNF2 HUM2)	79	EVQLVESGGGLVQPGGSLRLSCAASGFTFSEPSGYTYTIGWFRQAP GKGREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLQMNSLK PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSS
TNF17(TNF2 HUM3)	80	EVQLVESGGGLVQPGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKGREFVARIYWSSGLTYYADSVKGRFTISRDNAKNTVDLQMNSLK PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSS
TNF18(TNF2 HUM4)	81	EVQLVESGGGLVQPGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKGREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLQMNSLR PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSS
TNF19(TNF2 HUM5)	82	EVQLVESGGGLVQPGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKGREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLQMNSLK PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTLVTVSS
TNF20(TNF3 HUM1)	83	EVQLVESGGGLVQPGGSLRLSCAASGRSLSNYYMGWFRQAPGKGRE LLGNISWRGYNIYYKDSVKGRFTISRDDSKNTIYLQMNSLKPEDTA VYYCAASILPLSDDPGWNTYWGQGTQVTVSS
TNF21(TNF3 HUM2)	84	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYYMGWFRQAPGKGRE LLGNISWRGYNIYYKDSVKGRFTISRDDSKNTIYLQMNSLKPEDTA VYYCAASILPLSDDPGWNTYWGQGTQVTVSS
TNF22(TNF3 HUM3)	85	EVQLVESGGGLVQPGGSLRLSCAASGRSLSNYYMGWFRQAPGKGRE LLGNISWRGYNIYYKDSVKGRFTISRDDSKNTIYLQMNSLKTEDTA VYYCAASILPLSDDPGWNTYWGQGTQVTVSS
TNF23(TNF3 HUM4)	86	EVQLVESGGGLVQPGGSLRLSCAASGRSLSNYYMGWFRQAPGKGRE LLGNISWRGYNIYYKDSVKGRFTISRDDSKNTIYLQMNSLKPEDTA VYYCAASILPLSDDPGWNTYWGQGTLVTVSS
ALB3(ALB1 HUM1)	87	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKEPE WVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTA VYYCTIGGSLSRSSQGTQVTVSS
ALB4(ALB1 HUM2)	88	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSFGMSWVRQAPGKEPE WVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTA VYYCTIGGSLSRSSQGTQVTVSS
ALB5(ALB1 HUM3)	89	EVQLVESGGGLVQPGGSLRLSCAASGFTFRSFGMSWVRQAPGKGLE WVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTA VYYCTIGGSLSRSSQGTQVTVSS

1199

Tabla 26

Nanocuerpo	Tiempo de inducción	Rendimiento (mg/L)
TNF13	ON/28°C	8.8
TNF14	ON/28°C	7
TNF15	Corto/37°C	7.6
TNF16	Corto/37°C	8.7
TNF17	Corto/37°C	7.2
TNF18	Corto/37°C	4.8
TNF19	Corto/37°C	8
TNF20	ON/28°C	3.5
TNF21	ON/28°C	7.5
TNF22	ON/28°C	6
TNF23	ON/28°C	2.8
ALB3	ON/28°C	11.8
ALB4	ON/28°C	9
ALB5	ON/28°C	11.7

Tabla 27

ensayo: *L929s + Act D (5000c/w)*

TNF: *TNFα humano @ 0.5ng/ml*

	EC_{50} en nM			relative potency	
Nanocuerpo	media	desv.est	#	media	desv.est
TNF1	**0.707**	**0.265**	**14**	**0.015**	**0.007**
TNF13	0.988	0.014	3	0.014	0.003
TNF14	0.981	0.007	3	0.014	0.003
TNF2	**1.412**	**0.622**	**14**	**0.007**	**0.002**
TNF15	1.669	1.253	4	0.002	0.000
TNF16	1.898	0.192	4	0.005	0.001
TNF17	3.023	0.562	4	0.001	0.001
TNF18	1.508	0.481	4	0.004	0.001
TNF19	2.191	0.941	4	0.001	0.001
TNF3	**0.224**	**0.133**	**14**	**0.048**	**0.019**
TNF20	0.380	0.080	3	0.035	0.005
TNF21	0.889	0.019	3	0.015	0.003
TNF22	0.303	0.005	3	0.044	0.011
TNF23	0.3	0.011	3	0.044	0.011
Enbrel	0.009	0.005	45	1.002	0.011
Humira	0.079	0.043	39	0.097	0.069
Remicade	0.083	0.037	45	0.103	0.058

Tabla 28

%	Sin tratar	TA	37C	50C	60C	70C	80C	90C
TNF13	100	104	99	98	99	84	93	93
TNF14	100	98	101	95	99	96	99	90
TNF15	100	100	91	99	95	90	59	46
TNF16	100	97	102	101	94	101	58	48
TNF17	100	102	98	100	90	90	69	59
TNF18	100	100	101	97	91	93	63	50
TNF19	100	102	111	98	92	91	60	49
TNF20	100	94	93	93	93	92	85	67
TNF21	100	98	99	101	98	96	36	40
TNF22	100	102	101	105	99	93	25	31
TNF23	100	98	97	99	97	98	87	55
ALB3	100	100	99	98	25	18	60	62
ALB4	100	100	100	100	99	29	61	55
ALB5	100	100	100	99	94	32	61	48

Tabla 29

Nombre	SEC ID NO	Secuencia
TNF1-9GS-ALB1-9GS-TNF1(TNF24)	90	QVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTA LYYCARSPSGFNRGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQP GNSLRLSCAASGFTFRSFGMSWVRQAPGKEPEWVSSISGSGSDTLY ADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAVYYCTIGGSLSRSS QGTQVTVSSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGFT FSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDN AKNTLYLQMNSLKPEDTALYYCARSPSGFNRGQGTQVTVSS
TNF2-9GS-TNF2-9GS-ALB1(TNF25)	91	QVQLVESGGGLVQAGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKEREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLLMNSLK PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSSGGGGSGGGS EVQLVESGGGLVQAGGSLRLSCAASGRTFSEPSGYTYTIGWFRQAP GKEREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLLMNSLK PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSSGGGGSGGGS EVQLVESGGGLVQPGNSLRLSCAASGFTFRSFGMSWVRQAPGKEPE WVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTA VYYCTIGGSLSRSSQGTQVTVSS
TNF3-9GS-ALB1-9GS-TNF3(TNF26)	92	EVQLVESGGGLVQAGGSLSLSCSASGRSLSNYYMGWFRQAPGKERE LLGNISWRGYNIYYKDSVKGRFTISRDDAKNTIYLQMNRLKPEDTA VYYCAASILPLSDDPGWNTYWGQGTQVTVSSGGGGSGGGSEVQLVE SGGGLVQPGNSLRLSCAASGFTFRSFGMSWVRQAPGKEPEWVSSIS GSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAVYYCTI GGSLSRSSQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQAGGSLSL SCSASGRSLSNYYMGWFRQAPGKERELLGNISWRGYNIYYKDSVKG RFTISRDDAKNTIYLQMNRLKPEDTAVYYCAASILPLSDDPGWNTY WGQGTQVTVSS
TNF1-30GS-TNF1-9GS-ALB1(TNF27)	93	QVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTA LYYCARSPSGFNRGQGTQVTVSSGGGGSGGGSGGGGSGGGGSGGG GSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQ APGKGLEWVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNS LKPEDTALYYCARSPSGFNRGQGTQVTVSSGGGGSGGGSEVQLVES GGGLVQPGNSLRLSCAASGFTFRSFGMSWVRQAPGKEPEWVSSISG SGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAVYYCTIG GSLSRSSQGTQVTVSS
TNF3-30GS-TNF3-9GS-ALB1(TNF28)	94	EVQLVESGGGLVQAGGSLSLSCSASGRSLSNYYMGWFRQAPGKERE LLGNISWRGYNIYYKDSVKGRFTISRDDAKNTIYLQMNRLKPEDTA VYYCAASILPLSDDPGWNTYWGQGTQVTVSSGGGGSGGGSGGGGS GGGGSGGGSSGGGGSEVQVVESGGGLVQAGGSLSLSCSASGRSLSN YYMGWFRQAPGKERELLGNISWRGYNIYYKDSVKGRFTISRDDAKN TIYLQMNRLKPEDTAVYYCAASILPLSDDPGWNTYWGQGTQVTVSS GGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFRSFGMSWV RQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQM NSLKPEDTAVYYCTIGGSLSRSSQGTQVTVSS
TNF30-9GS-ALB8-9GS-TNF30 (TNF60)	417	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTA VYYCARSPSGFNRGQGTLVTVSSggggsgggsEVQLVESGGGLVQP GNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLY ADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSS QGTLVTVSSggggsgggsEVQLVESGGGLVQPGGSLRLSCAASGFT FSDYWMYWVRQAPGKGLEWVSEINTNGLITKYPDSVKGRFTISRDN AKNTLYLQMNSLRPEDTAVYYCARSPSGFNRGQGTLVTVSS

1201

TNF33-9GS-ALB8-9GS-TNF33 (TNF62)	418	EVQLVESGGGLVQPGGSLRLSCAASGRSLSNYYMGWFRQAPGKGRE LLGNISWRGYNIYYKDSVKGRFTISRDDSKNTIYLQMNSLRPEDTA VYYCAASILPLSDDPGWNTYWGQGTLVTVSSggggsgggsEVQLVE SGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSIS GSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTI GGSLSRSSQGTLVTVSSggggsgggsEVQLVESGGGLVQPGGSLRL SCAASGRSLSNYYMGWFRQAPGKGRELLGNISWRGYNIYYKDSVKG RFTISRDDSKNTIYLQMNSLRPEDTAVYYCAASILPLSDDPGWNTY WGQGTLVTVSS

Tabla 30

ID	Formato
TNF24	TNF1-9GS-ALB1-9GS-TNF1
TNF25	TNF2-9GS-TNF2-9GS-ALB1
TNF26	TNF3-9GS-ALB1-9GS-TNF3
TNF27	TNF1-30GS-TNF1-9GS-ALB1
TNF28	TNF3-30GS-TNF3-9GS-ALB1

Tabla 31

Nanocuerpo	Tiempo de inducción	Rendimiento (mg/L)
TNF24	ON/28°C	1.7
TNF25	corto/37°C	0.445
TNF26	corto/37°C	0.167
TNF27	ON/28°C	2.2
TNF28	corto/37°C	1

Tabla 32

ensayo: *L929s + Act D (5000c/w)*

TNF: *TNFa* humano *@ 0.5ng/ml*

	EC_{50} en nM			potencia relativa	
Nanocuerpo	media	desv.est	#	media	desv.est
TNF24	0.011	0.003	11	0.878	0.248
TNF25	0.018	0.008	14	0.603	0.243
TNF26	0.020	0.009	14	0.583	0.210
TNF27	0.012	0.003	3	0.810	0.037
TNF28	0.021	0.008	6	0.548	0.360
Enbrel	0.009	0.005	45	1.002	0.011
Humira	0.079	0.043	39	0.097	0.069
Remicade	0.083	0.037	45	0.103	0.058

1202

Tabla 33

Albúmina humana	KD (nM)	ka (1/Ms)	kd (1/s)
6A6 (ALB1)	0,57	1,11E+6	6,30E-4
1C2-GS-6A6-GS-1C2 (TNF24)	11	2,26E+05	2,48E-03
1G5-GS-1G5-GS-6A6 (TNF25)	7,2	2,91E+05	2,10E-03
5F10-GS-6A6-GS-5F10 (TNF26)	7,3	2,81E+05	2,05E-03
1C2-GS6-1C2-GS-6A6 (TNF27)	8,9	3,19E+05	2,84E-03
5F10-GS6-5F10-GS-6A6 (TNF28)	14	1,55E+05	2,13E-03

Tabla 34

%	Sin tratar	TA	37C	50C	60C	70C	80C	90C
TNF24	100	100	99	98	5	3	8	18
TNF25	100	nd	103	102	95	5	4	6
TNF26	100	109	115	112	107	10	8	10
TNF27	100	102	103	102	22	9	26	34
TNF28	100	97	99	99	66	3	6	10

1203

Tabla 35

Nombre	SEC ID NO	Secuencia
TNF29(TNF1 HUM1)	95	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLKPEDTA VYYCARSPSGFNRGQGTLVTVSS
TNF30(TNF1 HUM2)	96	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMYWVRQAPGKGLE WVSEINTNGLITKYPDSVKGRFTISRDNAKNTLYLQMNSLRPEDTA VYYCARSPSGFNRGQGTLVTVSS
TNF31(TNF2 HUM1)	97	EVQLVESGGGLVQPGGSLRLSCAASGFTFSEPSGYTYTIGWFRQAP GKGREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLQMNSLR PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTQVTVSS
TNF32(TNF2 HUM2)	98	EVQLVESGGGLVQPGGSLRLSCAASGFTFSEPSGYTYTIGWFRQAP GKGREFVARIYWSSGLTYYADSVKGRFTISRDIAKNTVDLQMNSLR PEDTAVYYCAARDGIPTSRSVGSYNYWGQGTLVTVSS
TNF33(TNF3 HUM1)	99	EVQLVESGGGLVQPGGSLRLSCAASGRSLSNYYMGWFRQAPGKGRE LLGNISWRGYNIYYKDSVKGRFTISRDDSKNTIYLQMNSLRPEDTA VYYCAASILPLSDDPGWNTYWGQGTLVTVSS
ALB6(ALB1 HUM1)	100	EVQLVESGGGLVQPGNSLRLSCAASGFTFRSFGMSWVRQAPGKGLE WVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTA VYYCTIGGSLSRSSQGTLVTVSS
ALB7(ALB1 HUM2)	101	EVQLVESGGGLVQPGNSLRLSCAASGFTFRSFGMSWVRQAPGKGLE WVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTA VYYCTIGGSLSRSSQGTLVTVSS
ALB8(ALB1 HUM3)	102	EVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLE WVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTA VYYCTIGGSLSRSSQGTLVTVSS
ALB9(ALB1 HUM4)	103	EVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLE WVSSISGSGSDTLYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTA VYYCTIGGSLSRSSQGTLVTVSS
ALB10(ALB1 HUM5)	104	EVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLE WVSSISGSGSDTLYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTA VYYCTIGGSLSRSGQGTLVTVSS

Tabla 36

Nanobody™	Tiempo de inducción	Rendimiento
TNF29	ON/28C	2.1 mg/L
TNF30	ON/28C	2.7 mg/L
TNF31	ON/28C	2 mg/L
TNF32	ON/28C	1.5 mg/L
TNF33	ON/28C	0.5 mg/L

1204

Tabla 37

ensayo: *L929s + Act D (5000c/w)*

TNF: *TNFa* humano *@ 0.5ng/ml*

	EC_{50} en nM			potencia relativa	
V_{HH}	media	desv.est	#	media	desv.est
TNF1	**0.707**	**0.265**	**14**	**0.015**	**0.007**
TNF13	0.988	0.014	3	0.014	0.003
TNF14	0.981	0.007	3	0.014	0.003
TNF29	1.336		1	0.013	
TNF30	0.985		1	0.017	
TNF2	**1.412**	**0.622**	**14**	**0.007**	**0.002**
TNF15	5.896	1.253	4	0.002	0.000
TNF16	2.422	0.192	4	0.005	0.001
TNF17	7.555	0.562	4	0.001	0.001
TNF18	3.134	0.481	4	0.004	0.001
TNF19	7.372	0.941	4	0.001	0.001
TNF31	2.195		1	0.008	
TNF32	2.506		1	0.007	
TNF3	**0.224**	**0.133**	**14**	**0.048**	**0.019**
TNF20	0.380	0.080	3	0.035	0.005
TNF21	0.889	0.019	3	0.015	0.003
TNF22	0.303	0.005	3	0.004	0.011
TNF23	0.3	0.011	3	0.04	0.011
TNF33	0.3		1	0.057	
Enbrel	0.009	0.005	45	1.002	0.011
Humira	0.079	0.043	39	0.097	0.069
Remicade	0.083	0.037	45	0.103	0.058

Tabla 38

	sin tratar	TA	37C	50C	60C	70C	80C	90C
TNF29	100	100	100	100	100	96	91	89
TNF30	100	100	100	99	100	96	92	89
TNF31	100	100	100	98	91	84	56	43
TNF32	100	99	98	97	87	78	45	39
TNF33	100	98	97	97	94	91	79	49

1205

Tabla 39

ensayo: *alphaKYM (10000c/w)*
TNF: *TNFa humano @ 1ng/ml*

Nanocuerpo	EC_{50} en nM
TNF1	2.466
TNF2	4.236
TNF3	0.655
TNF4	0.069
TNF5	0.008
TNF6	0.121
TNF7	0.009
TNF8	0.013
TNF9	0.020
Enbrel	0.040
Humira	0.103
Remicade	0.100

Resultados del Documento WO 04/41862

Nanocuerpo	SEC ID No	EC_{50} en nM
1A	1	100
3E	4	12
3G	5	20
Remicade		0,080

Tabla 40

M13 rev	SEC ID NO: 421	GGATAACAATTTCACACAGG
Rev 9GlySer L108	SEC ID NO: 422	TCAGTAACCTGGATCCGCCACCGCTGCCTCCACCGCCTGAGGAGACGGTGACCAG
For GS/corto	SEC ID NO: 423	AGGTTACTGAGGATCCGAGGTGCAGCTGGTGGAGTCTGG
Rev 15BspEI L108	SEC ID NO: 424	TCAGTAACCTTCCGGAACCGCCACCGCCTGAGGAGACGGTGACAAG
For BspEI	SEC ID NO: 425	AGGTTACTGATCCGGAGGCGGTAGCGAGGTGCAGCTGGTGGAGTCTGG
M13 para	SEC ID NO: 426	CACGACGTTGTAAAACGAC

Tabla 41

Cebador Inverso		Secuencia
PiRevhumNot/a40c (Not1)	SEC ID NO: 427	ATGGTGGTGTGCGGCCGCCTATTATGAGGAGACGGTGACCAGG
Cebador delantero		
Pi2for (Xho1)	SEC ID NO: 428	AGGGGTATCTCTCGAGAAAAGAGAGGTGCAGCTGGTGGAGTCTGG

Tabla 42

TNFα humano	EC_{50} en nM		
VHH	media	desv.est	Número de ensayos
TNF60	0.010	0.002	6
Enbrel	0.014	0.009	33
Humira	0.141	0.074	33
Remicade	0.120	0.037	33

Tabla 43

		albúmina humana	albúmina Rheus
TNF60	K_D (nM)	24,4	24,1
	k_{on} (1/Ms)	2,05E+05	2,09E+05
	k_{off} (1/s)	5,02E-03	5,04E-03
TNF24	K_D (nM)	11	Nd
	k_{on} (1/Ms)	2.26E+05	Nd
	k_{off} (1/s)	2.48E-03	Nd

Tabla 44

PiForLong	SEC ID NO: 429	GCTAAAGAAGAAGGGGTATCTCTCGAGAAAAGAGAGGTGCAGCTGGTGGAGTCTGG
Rev_30GlySer_L108	SEC ID NO: 430	TCAGTAACCTGGATCCCCCGCCACCGCTGCCTCCACCGCCGCTACCCCCGCCACCGC TGCCTCCACCGCCTGAGGAGACGGTGACAAG
For_GlySer	SEC ID NO: 431	ACGTTACTGAGGATCCGGCGGTGGAGGCAGCGGTGGCGGGGGCTAGCGAGGTGCAGCTGGTGGAGTCTGG
PiRevCys1hum	SEC ID NO: 432	ATGGTGGTGTGAATTCTTATTAGCAGGAGACGGTGACAAGG
PiRevCys2hum	SEC ID NO: 433	ATGGTGGTGTGAATTCTTATTAGCAACCTCCACCTGAGGAGACGGTGACAAGG
AOXIFor	SEC ID NO: 434	GACTGGTTCCAATTGACAAGC
AOXIRev	SEC ID NO: 435	GCAAATGGCATTCTGACATCC

Tabla 45

TNFα humano

	EC_{50} en nM		
VHH	media	desv.est	Número de ensayos
TNF1	0.748	0.153	27
TNF55-PEG40	0.004	0.001	8
TNF55-PEG60	0.004	0.002	6
TNF55-Biotina	0.012	0.003	5
TNF56-PEG40	0.006	0.003	57
TNF56-PEG60	0.005	0.003	7
TNF56-Biotina	0.017	0.009	13
Enbrel	0.013	0.006	71
Humira	0.127	0.058	67
Remicade	0.144	0.061	67

Tabla 46

	DS534 P4	DS592 P4	DS605 P3	KM05-179 P4
p[IC]50				
Etanercept	9.55	9.49	9.51	9.51
Accipiter	9.88	9.44	9.37	9.27
pM				
Etanercept	282	324	309	309
Accipiter	132	363	427	537

Tabla 47

	Conteo WBC total ($x10^6$/mL)	
Dosis por Grupo	ALX0071	Etanercept
Vehículo CMC	0.86 ± 0.09	
0.1 µg TNFα humano	3.72 ± 0.21	
0.0625 mg/kg	3.03 ± 0.6	-
0.125 mg/kg	1.23 ± 0.3**	2.40 ± 0.39
0.25 mg/kg	1.37 ± 0.17*	2.47 ± 0.54
0.5 mg/kg	-	2.19 ± 0.10

*P<0.05, **P<0.01 contra 0.1 µg TNFα humano

Aquellos expertos en la técnica reconocerán, o serán capaces de valorar usando no más que la experimentación de rutina, muchas equivalentes a las modalidades específicas de la invención descrita en este documento. Tales equivalentes están propuestos para estar abarcados por las siguientes reivindicaciones.

Todas las referencias descritas en este documento, están incorporadas por referencia en su totalidad.

1209

NOVEDAD DE LA INVENCIÓN

Habiéndose descrito la presente se considera como novedad, y por lo tanto, se reclama como propiedad lo contenido en las siguientes:

REIVINDICACIONES

1. Nanocuerpo contra TNF-alfa, el cual consiste de 4 regiones de estructura (FR1 hasta FR4 respectivamente) y 3 regiones que determinan la complementariedad (CDR1 hasta CDR3 respectivamente), caracterizado porque:

d) CDR1 comprende:

- la secuencia de aminoácido DYWMY; o

- una secuencia de aminoácido que tiene, al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido DYWMY; o

- secuencias de aminoácido que tienen solamente 1 diferencia de aminoácido con la secuencia de aminoácido DYWMY;

y

e) CDR2 comprende:

- la secuencia de aminoácido EINTNGLITKYPDSVKG; o

- una secuencia de aminoácido que tiene, al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido EINTNGLITKYPDSVKG; o

- secuencias de aminoácidos que tienen 2 ó solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido EINTNGLITKYPDSVKG; y

f) CDR3 comprende:

- la secuencia de aminoácido SPSGFN; o

- una secuencia de aminoácido que tiene, al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido SPSGFN; o

- secuencias de aminoácido que tienen solamente 1 diferencia de aminoácido con la secuencia de aminoácido SPSGFN.

2. Nanocuerpo de conformidad con la reivindicación 1, caracterizado porque CDR1 comprende la secuencia de aminoácido DYWMY.

3. Nanocuerpo de conformidad con la reivindicación 1, caracterizado porque CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG.

4. Nanocuerpo de conformidad con la

1211

reivindicación 1, caracterizado porque CDR3 comprende la secuencia de aminoácido SPSGFN.

5. Nanocuerpo de conformidad con la reivindicación 1, caracterizado porque:

- CDR1 comprende la secuencia de aminoácido DYWMY; y CDR3 comprende la secuencia de aminoácido SPSGFN; o

- CDR1 comprende la secuencia de aminoácido DYWMY; y CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG; o

- CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG; y CDR3 comprende la secuencia de aminoácido SPSGFN

6. Nanocuerpo de conformidad con la reivindicación 1, caracterizado porque CDR1 comprende la secuencia de aminoácido DYWMY; y CDR3 comprende la secuencia de aminoácido SPSGFN.

7. Nanocuerpo de conformidad con la reivindicación 1, caracterizado porque CDR1 comprende la secuencia de aminoácido DYWMY; CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG y CDR3 comprende la secuencia de aminoácido SPSGFN.

8. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 1-7, caracterizado porque

- cualquier sustitución de aminoácido es una

sustitución de aminoácido conservativa; y/o

- dicha secuencia de aminoácido solamente contiene sustituciones de aminoácido, y no supresiones o inserciones de aminoácido, comparada con la(s) secuencia(s) de aminoácido anterior(es).

9. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 1-8, caracterizado porque es un Nanocuerpo humanizado.

10. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 1-9, caracterizado porque es un Nanocuerpo clase GLEW.

11. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 1-9, caracterizado porque contiene un residuo de arginina (R) en la posición 103.

12. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 1-9, caracterizado porque es un Nanocuerpo clase GLEW.

13. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 1-9, caracterizado porque es un Nanocuerpo clase GLEW con un residuo de arginina (R) en la posición 103.

14. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 1-13, caracterizado porque tiene al menos 80%, o al menos 90%, o al menos 95%, o al menos 99% de identidad de secuencia (como se define aquí) con una de

la secuencia de aminoácidos de las SEC ID NOS. 52 (TNF1), 76 (TNF13), 77 (TNF14), 95 (TNF29) ó 96 (TNF30).

15. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 1-14, caracterizado porque contiene un residuo leucina (L) en la posición 108.

16. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 1-8, caracterizado porque tiene una relación Koff para TNF mejor de 2.10-3 (1/s), preferiblemente mejor de 1.10-3 (1/s); o una variante humanizada de tal Nanocuerpo.

17. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 1-8, caracterizado porque tiene un valor EC50 en el ensayo a base de células, usando Células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que el valor EC50 del Nanocuerpo VHH 3E (SEC ID NO:4) del documento WO 04/041862 en el mismo ensayo; o una variante humanizada de tal Nanocuerpo.

18. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 1-8, caracterizado porque tiene un valor EC50 en el ensayo a base de células, usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor de 5nM; o una variante humanizada de tal Nanocuerpo.

19. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 1-8, caracterizado porque tiene un

valor EC50 en el ensayo a base de células, usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor de 3nM; o una variante humanizada de tal Nanocuerpo.

20. Nanocuerpo contra TNF-alfa, el cual consiste de 4 regiones de estructura (FR1 hasta FR4 respectivamente) y 3 regiones que determinan la complementariedad (CDR1 hasta CDR3 respectivamente), caracterizado porque

d) CDR1 es:

- la secuencia de aminoácido DYWMY; o

- una secuencia de aminoácido que tiene, al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido DYWMY; o

- secuencias de aminoácido que tienen solamente 1 diferencia de aminoácido con la secuencia de aminoácido DYWMY;

y en la cual:

e) CDR2 es:

- la secuencia de aminoácido EINTNGLITKYPDSVKG; o

- una secuencia de aminoácido que tiene, al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido

EINTNGLITKYPDSVKG; o

- secuencias de aminoácidos que tienen 2 ó solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido EINTNGLITKYPDSVKG;

y en la cual

f) CDR3 es:

- la secuencia de aminoácido SPSGFN; o

- una secuencia de aminoácido que tiene, al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido SPSGFN; o

- secuencias de aminoácidos que tienen solamente 1 diferencia de aminoácido con la secuencia de aminoácido SPSGFN.

21. Nanocuerpo de conformidad con la reivindicación 20, caracterizado porque CDR1 es la secuencia de aminoácido DYWMY.

22. Nanocuerpo de conformidad con la reivindicación 20, caracterizado porque CDR2 es la secuencia de aminoácido EINTNGLITKYPDSVKG.

23. Nanocuerpo de conformidad con la reivindicación 20, caracterizado porque CDR3 es la secuencia de aminoácido SPSGFN.

24. Nanocuerpo de conformidad con la

reivindicación 20, caracterizado porque:

- CDR1 es la secuencia de aminoácido DYWMY; y CDR3 es la secuencia de aminoácido SPSGFN; o

- CDR1 es la secuencia de aminoácido DYWMY; y CDR2 es la secuencia de aminoácido EINTNGLITKYPDSVKG; o

- CDR2 es la secuencia de aminoácido EINTNGLITKYPDSVKG; y CDR3 es la secuencia de aminoácido SPSGFN.

25. Nanocuerpo de conformidad con la reivindicación 20, caracterizado porque CDR1 es la secuencia de aminoácido DYWMY; y CDR3 es la secuencia de aminoácido SPSGFN.

26. Nanocuerpo de conformidad con la reivindicación 20, caracterizado porque CDR1 es la secuencia de aminoácido DYWMY; CDR2 es la secuencia de aminoácido EINTNGLITKYPDSVKG y CDR3 es la secuencia de aminoácido SPSGFN.

27. Un Nanocuerpo de conformidad con cualquiera de las reivindicaciones 20-26, caracterizado porque:

- cualquier sustitución de aminoácido es preferiblemente una sustitución de aminoácido conservativo; y/o

- dicha secuencia de aminoácido preferiblemente solamente contiene sustituciones de aminoácido, y no supresiones o inserciones de aminoácido, comparada con

la(s) secuencia(s) de aminoácido anterior(es).

28. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 20-27, caracterizado porque es un Nanocuerpo humanizado.

29. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 20-27, caracterizado porque es un Nanocuerpo clase GLEW.

30. Un Nanocuerpo de conformidad con cualquiera de las reivindicaciones 20-27, caracterizado porque contiene un residuo de arginina (R) en la posición 103.

31. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 20-27, Nanocuerpo de conformidad con cualquiera de las reivindicaciones 1-8, caracterizado porque es un Nanocuerpo clase GLEW con un residuo de arginina (R) en la posición 103.

32. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 20-27, caracterizado porque tiene al menos 80%, o al menos 90%, o al menos 95%, o al menos 99% de identidad de secuencia (como se define aquí) con una de la secuencia de aminoácidos de las SEC ID NOS. 52 (TNF1), 76 (TNF13), 77 (TNF14), 95 (TNF29) ó 96 (TNF30).

33. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 20-27, caracterizado porque contiene un residuo leucina (L) en la posición 108.

34. Nanocuerpo de conformidad con cualquiera de

las reivindicaciones 20-27, caracterizado porque tiene una relación Koff de TNF mejor de 2.10-3 (1/s), preferiblemente mejor de 1.10-3 (1/s); o una variante humanizada de tal Nanocuerpo.

35. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 20-27, caracterizado porque tiene un valor EC50 en el ensayo a base de células, usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que el valor EC50 del Nanocuerpo VHH 3E (SEC ID NO:4) del documento WO 04/041862 en el mismo ensayo o una variante humanizada de tal Nanocuerpo.

36. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 20-27, caracterizado porque tiene un valor EC50 en el ensayo a base de células, usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor de 5nM; o una variante humanizada de tal Nanocuerpo.

37. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 20-27, caracterizado porque tiene un valor EC50 en el ensayo a base de células, usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor de 3nM; o una variante humanizada de tal Nanocuerpo.

38. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 1 -37, caracterizado porque es elegido

del grupo que consiste de TNF 13 (SEC ID NO: 76), TNF 14 (SEC ID NO: 77), TNF 29 (SEC ID NO: 95) y TNF 30 (SEC ID NO:96).

39. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 1-38, caracterizado porque es TNF 30 (SEC ID NO: 96).

40. Proteína o polipéptido, caracterizados porque comprenden o consisten esencialmente de un Nanocuerpo de conformidad con cualquiera de las reivindicaciones 1-39.

41. Proteína o polipéptido de conformidad con la reivindicación 40, caracterizados porque comprenden o consisten esencialmente de al menos un Nanocuerpo de conformidad con cualquiera de las reivindicaciones 1-39.

42. Proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 40-41, caracterizados porque comprenden dos Nanocuerpos de conformidad con cualquiera de las reivindicaciones 1-39.

43. Proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 40-42, caracterizados porque comprenden dos Nanocuerpos de conformidad con cualquiera de las reivindicaciones 1-39, y el cual es tal que dicha proteína o polipéptido, después del enlace a un trímero de TNF, es capaz de inhibir o reducir la reticulación del receptor de TNF que es mediada por dicho trímero de TNF y/o la señal de transducción que es mediada

por tal reticulación del receptor.

44. Proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 40-42, caracterizados porque comprenden dos Nanocuerpos de conformidad con cualquiera de las reivindicaciones 1-39, y el cual es capaz de enlazarse intramolecularmente con al menos dos sitios de enlace al receptor de TNF en un trímero de TNF.

45. Proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 42-44, caracterizados porque comprenden dos Nanocuerpos de conformidad con cualquiera de las reivindicaciones 1-39, los cuales son directamente ligados entre sí o enlazados entre sí vía un enlazador.

46. Proteína o polipéptido de conformidad con la reivindicación 45, caracterizados porque el enlazador es una secuencia de aminoácido.

47. Proteína o polipéptido de conformidad con la reivindicación 46, caracterizados porque el enlazador es una secuencia de aminoácido que comprende al menos 14 aminoácidos, más preferiblemente al menos 17 aminoácidos, tal como aproximadamente 20-40 aminoácidos.

48. Proteína o polipéptido de conformidad con la reivindicación 47, caracterizados porque el enlazador comprende residuos de glicina y serina.

49. Proteína o polipéptido de conformidad con

cualquiera de las reivindicaciones 42-48, caracterizados porque comprende o consiste esencialmente del polipéptido TNF 7 (SEC ID NO: 73), en los cuales ambos Nanocuerpos TNF 1 han sido humanizados.

50. Proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 42-49, caracterizados porque comprenden o consisten esencialmente del polipéptido TNF 55 (SEC ID NO: 419) o TNF 56 (SEC ID NO: 420).

51. Proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 40-49, caracterizados porque son pegilados.

52. Proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 40-49, caracterizados porque además comprenden al menos un Nanocuerpo dirigido contra albúmina de suero humana.

53. Proteína o polipéptido de conformidad con la reivindicación 52, caracterizados porque al menos un Nanocuerpo dirigido contra albúmina de suero humana tiene las secuencias CDR presentes en ALB 8 (SEC ID NO: 102).

54. Proteína o polipéptido de conformidad con la reivindicación 42, caracterizados porque comprenden dos Nanocuerpos de conformidad con cualquiera de las reivindicaciones 1-39, los cuales además comprenden al menos un Nanocuerpo dirigido contra albúmina de suero humana.

55. Proteína o polipéptido de conformidad con la reivindicación 54, caracterizados porque los dos Nanocuerpos de conformidad con cualquiera de las reivindicaciones 1-39 están ya sea directamente ligados con al menos un Nanocuerpo dirigido contra albúmina de suero humana, o están ligados con al menos un Nanocuerpo dirigido contra albúmina de suero humana vía un enlazador.

56. Proteína o polipéptido de conformidad con la reivindicación 55, caracterizados porque el enlazador es una secuencia de aminoácido.

57. Proteína o polipéptido de conformidad con la reivindicación 55, caracterizados porque el enlazador es una secuencia de aminoácido que comprende entre 3 y 40 residuos de aminoácidos, tal como entre 5 y 15 residuos de aminoácidos.

58. Proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 56 y 57, caracterizados porque el enlazador consiste de residuos de glicina y serina.

59. Proteína o polipéptido de conformidad con la reivindicación 54-58, caracterizados porque al menos un Nanocuerpo dirigido contra albúmina de suero humana tiene las secuencias CDR presentes en ALB 8 (SEC ID NO: 102).

60. Proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 54-59, caracterizados

porque al menos un Nanocuerpo dirigido contra albúmina de suero humana es un Nanocuerpo humanizado.

61. Proteína o polipéptido de conformidad con la reivindicación 60, caracterizados porque al menos un Nanocuerpo dirigido contra albúmina de suero humana es una variante humanizada del Nanocuerpo ALB 1 (SEC ID NO: 63).

62. Proteína o polipéptido de conformidad con la reivindicación 60, caracterizados porque al menos un Nanocuerpo dirigido contra albúmina de suero humana es elegido del grupo que consiste de ALB 3 (SEC ID NO: 87), ALB 4 (SEC ID NO: 88), ALB 5 (SEC ID NO: 89), ALB 6 (SEC ID NO: 100), ALB 7 (SEC ID NO: 101), ALB 8 (SEC ID NO: 102), ALB 9 (SEC ID NO: 103) y ALB 10 (SEC ID NO: 104).

63. Proteína o polipéptido de conformidad con la reivindicación 62, caracterizados porque al menos un Nanocuerpo dirigido contra albúmina de suero humana es ALB 8 (SEC ID NO: 102).

64. Proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 54-63, caracterizados porque comprenden o consisten esencialmente de dos Nanocuerpos los cuales son variantes humanizadas de un Nanocuerpo de conformidad con cualquiera de las reivindicaciones 1-39 y una variante humanizada del Nanocuerpo ALB 1 (SEC ID NO: 63).

65. Proteína o polipéptido de conformidad con

cualquiera de las reivindicaciones 54-64, caracterizados porque comprenden o consisten esencialmente del polipéptido TNF24 (SEC ID NO: 90), en los cuales tanto el Nanocuerpo TNF 1, así como también el Nanocuerpo ALB 1 ha sido humanizado.

66. Proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 54-65, caracterizados porque comprenden o consisten esencialmente de dos Nanocuerpos TNF 30 y un Nanocuerpo ALB 8.

67. Proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 54-66, caracterizados porque comprenden o consisten esencialmente del polipéptido TNF 60 (SEC ID NO: 417).

68. Nanocuerpo contra TNF-alfa, caracterizado porque es una variante humanizada de un Nanocuerpo contra TNF-alfa, en el cual el Nanocuerpo contra TNF-alfa tiene las secuencias siguientes secuencias de estructura: FR1: SEC ID NO: 130; FR2: SEC ID NO: 198; FR3: SEC ID NO: 266; y FR4: SEC ID NO: 334.

69. Nanocuerpo de conformidad con la reivindicación 68, caracterizado porque tiene las CDR que son tales que el Nanocuerpo tiene una relación Koff de TNF mejor de 2.10-3 (1/s), preferiblemente mejor de 1.10-3 (1/s).

70. Nanocuerpo de conformidad con la

reivindicación 68 o 69, caracterizado porque tiene CDRs que son tales que el Nanocuerpo tiene un valor EC50 en el ensayo a base de células, usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que el valor EC50 del Nanocuerpo VHH 3E (SEC ID NO:4) del documento WO 04/041862 en el mismo ensayo; y en particular mejor de 12nM, más en particular mejor de 5 nM, aún más en particular mejor de 3nM.

71. Nanocuerpo de conformidad con cualquiera de las reivindicaciones 68-70, caracterizado porque es dirigido contra el mismo epítope de TNF (es decir, el trímero de TNF) como TNF1.

72. Nanocuerpo caracterizado porque es dirigido contra el mismo epítope en el trímero de TNF-alfa como el Nanocuerpo TNF1 (SEC ID NO: 52).

73. Un Nanocuerpo caracterizado porque es dirigido contra el mismo epítope en el trímero de TNF-alfa como Nanocuerpo TNF3 (SEC ID NO: 60).

74. Nanocuerpo de conformidad con la reivindicación 73, caracterizado porque tiene CDRs que son tales que el Nanocuerpo tiene una relación Koff para TNF mejor de 2.10-3 (1/s), preferiblemente mejor de 1.10-3 (1/s).

75. Nanocuerpo de conformidad con la reivindicación 73 ó reivindicación 74, caracterizado porque

tiene CDRs que son tales que el Nanocuerpo tiene un valor EC50 en el ensayo a base de células, usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que el valor EC50 del Nanocuerpo VHH 3E (SEC ID NO:4) del documento WO 04/041862 en el mismo ensayo; y en particular mejor de 12nM, más en particular mejor de 5 nM, aún más en particular mejor de 3nM.

76. Nanocuerpo, caracterizado porque es una variante humanizada del Nanocuerpo TNF1 (SEC ID NO: 52).

77. Nanocuerpo de conformidad con la reivindicación 76, caracterizado porque tiene CDRs que son tales que el Nanocuerpo tiene una relación Koff para TNF mejor de 2.10-3 (1/s), preferiblemente mejor de 1.10-3 (1/s).

78. Nanocuerpo de conformidad con la reivindicación 76 ó reivindicaciones 77, caracterizado porque tiene CDRs que son tales que el Nanocuerpo tiene un valor EC50 en el ensayo a base de células, usando células KYM descritas en el Ejemplo 1, bajo 3), del documento WO 04/041862 que es mejor que el valor EC50 del Nanocuerpo VHH 3 E (SEC ID NO:4) del documento WO 04/041862 en el mismo ensayo; y en particular mejor de 12nM, más en particular mejor de 5 nM, aún más en particular mejor de 3nM.

79. Proteína o polipéptido, caracterizado porque comprende o consiste esencialmente de un Nanocuerpo de

conformidad con cualquiera de las reivindicaciones 68-78.

80. Proteína o polipéptido de conformidad con la reivindicación 79, caracterizados porque comprenden o consisten esencialmente de al menos un Nanocuerpo de conformidad con cualquiera de las reivindicaciones 68-78.

81. Proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 79-80, caracterizados porque comprenden dos Nanocuerpos de conformidad con cualquiera de las reivindicaciones 68-78.

82. Proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 79-81, caracterizados porque comprenden dos Nanocuerpos de conformidad con cualquiera de las reivindicaciones 68-78, y en los cuales dicha proteína o polipéptido, después del enlace a un trímero de TNF, es capaz de inhibir o reducir la reticulación del receptor de TNF que es mediada por dicho trímero de TNF y/o la señal de transducción que es mediada por tal reticulación del receptor.

83. Proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 79-81, caracterizados porque comprenden dos Nanocuerpos de conformidad con cualquiera de las reivindicaciones 68-78, y los cuales son capaz de enlazarse intramolecularmente con al menos dos sitios de enlace al receptor de TNF en un trímero de TNF.

84. Proteína o polipéptido de conformidad con

cualquiera de las reivindicaciones 81-83, caracterizados porque comprenden dos Nanocuerpos de conformidad con cualquiera de las reivindicaciones 68-78, los cuales son directamente ligados entre sí o enlazados entre sí vía un enlazador.

85. Proteína o polipéptido de conformidad con la reivindicación 84, caracterizados porque el enlazador es una secuencia de aminoácido.

86. Proteína o polipéptido de conformidad con la reivindicación 85, caracterizados porque el enlazador es una secuencia de aminoácido que comprende al menos 14 aminoácidos, más preferiblemente al menos 17 aminoácidos, tal como aproximadamente 20-40 aminoácidos.

87. Proteína o polipéptido de conformidad con la reivindicación 86, caracterizados porque el enlazador comprende residuos de glicina y serina.

88. Proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 81-83, caracterizados porque comprenden o consisten esencialmente del polipéptido TNF 7 (SEC ID NO: 73), en los cuales ambos Nanocuerpos TNF 1 han sido humanizados.

89. Proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 80-88, caracterizados porque son pegilado.

90. Proteína o polipéptido de conformidad con la

reivindicación 80, caracterizados porque comprenden dos Nanocuerpos de conformidad con cualquiera de las reivindicaciones 68-78, y en los cuales la proteína o polipéptido además comprenden al menos un Nanocuerpo dirigido contra albúmina de suero humana.

91. Proteína o polipéptido de conformidad con la reivindicación 90, caracterizados porque los dos Nanocuerpos de conformidad con cualquiera de las reivindicaciones 68-78 están ya sea directamente ligados con al menos un Nanocuerpo dirigido contra albúmina de suero humana, o están ligados con al menos un Nanocuerpo dirigido contra albúmina de suero humana vía un enlazador.

92. Proteína o polipéptido de conformidad con la reivindicación 91, caracterizados porque el enlazador es una secuencia de aminoácido.

93. Proteína o polipéptido de conformidad con la reivindicación 92, caracterizados porque el enlazador es una secuencia de aminoácido que comprenden entre 3 y 40 residuos de aminoácidos, tal como entre 5 y 15 residuos de aminoácidos.

94. Proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 92 ó 93, caracterizados porque el enlazador consiste de residuos de glicina y serina.

95. Proteína o polipéptido de conformidad con

1230

cualquiera de las reivindicaciones 90-94, caracterizados porque al menos un Nanocuerpo dirigido contra albúmina de suero humana tiene las secuencias CDR presentes en ALB 8 (SEC ID NO: 102).

96. Proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 90-95, caracterizados porque al menos un Nanocuerpo dirigido contra albúmina de suero humana es un Nanocuerpo humanizado.

97. Proteína o polipéptido de conformidad con la reivindicación 96, caracterizados porque al menos un Nanocuerpo dirigido contra albúmina de suero humana es una variante humanizada del Nanocuerpo ALB 1 (SEC ID NO: 63).

98. Proteína o polipéptido de conformidad con la reivindicación 97, caracterizados porque al menos un Nanocuerpo dirigido contra albúmina de suero humana es elegido del grupo que consiste de ALB 3 (SEC ID NO: 87), ALB 4 (SEC ID NO: 88), ALB 5 (SEC ID NO: 89), ALB 6 (SEC ID NO: 100), ALB 7 (SEC ID NO: 101), ALB 8 (SEC ID NO: 102), ALB 9 (SEC ID NO: 103) y ALB 10 (SEC ID NO: 104).

99. Proteína o polipéptido de conformidad con la reivindicación 98, caracterizados porque al menos un Nanocuerpo dirigido contra albúmina de suero humana es ALB 8 (SEC ID NO: 102).

100. Proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 81-99, caracterizados

porque comprenden o consisten esencialmente de dos Nanocuerpos los cuales son variantes humanizadas de un Nanocuerpo de conformidad con cualquiera de las reivindicaciones 65-67 y una variante humanizada del Nanocuerpo ALB 1 (SEC ID NO: 63).

101. Una proteína o polipéptido, caracterizados porque comprenden o consisten esencialmente de dos o más dominios variables de immunoglobulina (o fragmentos adecuados de los mismos) que son cada uno dirigidos contra epítopes en TNF-alfa (y en particular, del trímero de TNF-alfa) que caen en, y/o forman parte del(los) sitio(s) de enlace al receptor del trímero de TNF, de manera tal que dicho polipéptido, después del enlace a un trímero de TNF, es capaz de inhibir o reducir la reticulación del receptor de TNF que es mediada por dicho trímero de TNF y/o la señal de transducción que es mediada por tal reticulación del receptor.

102. Una proteína o polipéptido, caracterizados porque comprenden o consisten esencialmente de dos o más dominios variables de immunoglobulina (o fragmentos adecuados de los mismos) que son cada uno dirigidos contra epítopes en TNF-alfa (y en particular del trímero TNF-alfa) que cae en y/o forma parte del sitio(s) de enlace al receptor del trímero de TNF, de manera tal que dicho polipéptido es capaz del enlace intramolecular con al menos

dos sitios de enlace al receptor de TNF en un trímero de TNF.

103. Proteína o polipéptido de conformidad con la reivindicación 101 ó 102, caracterizados porque los dos dominios variables de inmunoglobulina son dirigidos contra el mismo epítope como Nanocuerpo TNF1 (SEC ID NO: 52).

104. Proteína o polipéptido de conformidad con la reivindicación 101 ó 102, caracterizados porque los dos o más dominios variables de inmunoglobulina son Nanocuerpos.

105. Secuencia de nucleótido o ácido nucleico, caracterizada porque codifica un Nanocuerpo, proteína o polipéptido de conformidad con cualquiera de las reivindicaciones precedentes.

106. Célula hospedera, caracterizada porque comprende una secuencia de nucleótido o ácido nucleico de conformidad con la reivindicación 105, o la cual expresa o es capaz de expresar un Nanocuerpo, proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 1-104.

107. Método para preparar un Nanocuerpo, proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 1-102, caracterizado porque comprende cultivar o mantener una célula hospedera de conformidad con la reivindicación 106, bajo condiciones tal que dicha célula hospedera produce o expresa un Nanocuerpo, proteína

o polipéptido de conformidad con cualquiera de las reivindicaciones 1-104; y la cual además comprende, aislar el Nanocuerpo, proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 1-104.

108. Composición farmacéutica, caracterizada porque comprende al menos, un Nanocuerpo, proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 1-104, y opcionalmente, al menos un portador farmacéuticamente aceptable.

109. Una formulación farmacéutica de conformidad con la reivindicación 108, o un Nanocuerpo, proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 1-104, caracterizada porque es para el tratamiento de al menos, un trastorno o enfermedad elegido del grupo que consiste de inflamación, artritis reumatoide, COPD, asma, enfermedad de Crohn, colitis ulcerativa, síndrome del intestino inflamatorio, esclerosis múltiple, enfermedad de Addison, hepatitis autoinmune, parotitis autoinmune, diabetes tipo I, epididimitis, glomerulonefritis, enfermedad de Graves, síndrome Guillain-Barre, enfermedad de Hashimoto, anemia hemolítica, lupus eritematoso sistémico, infertilidad masculina, esclerosis múltiple, miastenia gravis, pénfigo, psoriasis, fiebre reumática, artritis reumatoide, sarcoidosis, escleroderma, síndrome de Sjogren, espondiloartropatías, tiroiditis y

vasculitis.

110. Uso de un Nanocuerpo, proteína o polipéptido de conformidad con cualquiera de las reivindicaciones 1-104, en la preparación de un medicamento para el tratamiento de al menos, una enfermedad o trastorno elegido del grupo que consiste de inflamación, artritis reumatoide, COPD, asma, enfermedad de Crohn, colitis ulcerativa, síndrome del intestino inflamatorio, esclerosis múltiple, enfermedad de Addison, hepatitis autoinmune, parotitis autoinmune, diabetes tipo I, epididimitis, glomerulonefritis, enfermedad de Graves, síndrome Guillain-Barre, enfermedad de Hashimoto, anemia hemolítica, lupus eritematoso sistémico, infertilidad masculina, esclerosis múltiple, miastenia gravis, pénfigo, psoriasis, fiebre reumática, artritis reumatoide, sarcoidosis, escleroderma, síndrome de Sjogren, espondiloartropatías, tiroiditis y vasculitis.

111. Proteína o polipéptido, caracterizado porque tiene al menos 80%, o al menos 90%, o al menos 95%, o al menos 99% de identidad de secuencia (como se define aquí) con el polipéptido de TNF 60 (SEC ID NO: 417).

RESUMEN DE LA INVENCIÓN

La presente invención se refiere a Nanobodies™ (Nanocuerpos) mejorados contra el factor-alfa de necrosis del tumor (TNF-alfa), así como también a polipéptidos que comprenden o consisten esencialmente de uno o más de tales Nanocuerpos. La invención también se refiere a ácidos nucleicos que codifican tales Nanocuerpos y polipéptidos; a métodos para preparar tales Nanocuerpos y polipéptidos; a células hospederas que expresan o son capaces de expresar tales Nanocuerpos o polipéptidos; a composiciones que comprenden tales Nanocuerpos, polipéptidos, ácidos nucleicos o células hospederas; y a usos de tales Nanocuerpos, tales polipéptidos, tales ácidos nucleicos, tales células hospederas o tales composiciones, en particular, para propósitos profilácticos, terapéuticos o de diagnóstico, tales como los propósitos profilácticos, terapéuticos o de diagnóstico.

LISTADO DE SECUENCIA

<110> Ablynx N.V.

<120> Nanobodies™ (Nanocuerpos) mejorados contra el Factor-alfa del Factor Necrosis del Tumor

<130> ABL-027-PCT

<140> PCT/EP2006/004678
<141> 2006-05-17

<150> 60/682,332
<151> 2005-05-18

<160> 476

<170> PatentIn versión 3.3

<210> 1
<211> 26
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<220>
<221> CARACTERÍSTICAS_MISCELÁNEAS
<222> (11)..(11)
<223> Residuo Hallmark

<400> 1

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Xaa Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
20 25

<210> 2
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<220>
<221> CARACTERÍSTICAS_MISCELÁNEAS
<222> (2)..(2)
<223> Residuo Hallmark

<220>

<221> CARACTERÍSTICAS_MISCELÁNEAS
<222> (9)..(10)
<223> Residuo Hallmark

<220>
<221> CARACTERÍSTICAS_MISCELÁNEAS
<222> (12)..(12)
<223> Residuo Hallmark

<400> 2

Trp Xaa Arg Gln Ala Pro Gly Lys Xaa Xaa Glu Xaa Val Ala
1 5 10

<210> 3
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<220>
<221> CARACTERÍSTICAS_MISCELÁNEAS
<222> (21)..(22)
<223> Residuo Hallmark

<400> 3

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Xaa Xaa Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 4
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<220>
<221> CARACTERÍSTICAS_MISCELÁNEAS
<222> (1)..(2)
<223> Residuo Hallmark

<220>
<221> CARACTERÍSTICAS_MISCELÁNEAS
<222> (6)..(6)
<223> Residuo Hallmark

<400> 4

Xaa Xaa Gln Gly Thr Xaa Val Thr Val Ser Ser
1 5 10

<210> 5
<211> 26
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 5

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
20 25

<210> 6
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2
<400> 6

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Val Ala
1 5 10

<210> 7
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 7

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 8
<211> 13
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 8

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Ala
1 5 10

<210> 9
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 9

Trp Phe Arg Gln Ala Pro Gly Lys Gln Arg Glu Leu Val Ala
1 5 10

<210> 10
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 10

Trp Phe Arg Gln Ala Pro Gly Lys Gln Arg Glu Phe Val Ala
1 5 10

<210> 11
<211> 13
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 11

Trp Tyr Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ala
1 5 10

<210> 12
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 12

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 13
<211> 11
<212> PRT
<213> Artificial
<220>
<223> Elemento FR4

<400> 13

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 14
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 14

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> 15
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 15

Asp Tyr Trp Met Tyr
1 5

<210> 16
<211> 10
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 16

Glu Pro Ser Gly Tyr Thr Tyr Thr Ile Gly
1 5 10

<210> 17
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 17

Asn Tyr Tyr Met Gly
1 5

<210> 18
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 18

Val Ser Trp Met Tyr
1 5

<210> 19
<211> 8
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 19

Ala His Ser Val Tyr Thr Met Gly
1 5

<210> 20
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 20

Gly Tyr Tyr Met Gly
1 5

<210> 21
<211> 10

<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 21

Asp Tyr Ser Gly Tyr Thr Tyr Thr Val Gly
1 5 10

<210> 22
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 22

Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val Lys
1 5 10 15

Gly

<210> 23
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 23

Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 24
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 24

Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val Lys
1 5 10 15

Gly

<210> 25
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2
<400> 25

Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Val Asp Ser Val Lys
1 5 10 15

Gly

<210> 26
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2
<400> 26

Arg Ile Tyr Trp Ser Ser Gly Asn Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 27
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 27

Arg Ile Tyr Trp Ser Ser Ala Asn Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 28
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 28

7244

Ser Ile Ser Trp Arg Gly Asp Asn Thr Tyr Tyr Lys Glu Ser Val Lys
1 5 10 15

Gly

<210> 29
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 29

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Gly Ser Tyr Asn Tyr
1 5 10 15

<210> 30
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 30

Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
1 5 10

<210> 31
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 31

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Glu Ala Tyr Asn Tyr
1 5 10 15

<210> 32
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 32

Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Asn
1 5 10

<210> 33
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 33

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Glu Ser Tyr Asn Tyr
1 5 10 15

<210> 34
<211> 6
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 34

Ser Pro Ser Gly Phe Asn
1 5

<210> 35
<211> 6
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 35

Ser Pro Ser Gly Ser Phe
1 5

<210> 36
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 36

Ser Phe Gly Met Ser
1 5

<210> 37
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 37

Leu Asn Leu Met Gly
1 5

<210> 38
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 38

Ile Asn Leu Leu Gly
1 5

<210> 39
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 39

Asn Tyr Trp Met Tyr
1 5

<210> 40
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 40

Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 41
<211> 16
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 41

Thr Ile Thr Val Gly Asp Ser Thr Asn Tyr Ala Asp Ser Val Lys Gly
1 5 10 15

<210> 42
<211> 16
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 42

Thr Ile Thr Val Gly Asp Ser Thr Ser Tyr Ala Asp Ser Val Lys Gly
1 5 10 15

<210> 43
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 43

Ser Ile Asn Gly Arg Gly Asp Asp Thr Arg Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 44
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 44

Ala Ile Ser Ala Asp Ser Ser Thr Lys Asn Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 45
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 45

Ala Ile Ser Ala Asp Ser Ser Asp Lys Arg Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 46
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 46

Arg Ile Ser Thr Gly Gly Gly Tyr Ser Ty r Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly
<210> 47
<211> 13
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 47

Asp Arg Glu Ala Gln Val Asp Thr Leu Asp Phe Asp Tyr
1 5 10

<210> 48
<211> 6
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 48

Gly Gly Ser Leu Ser Arg
1 5

<210> 49
<211> 8
<212> PRT
<213> Artificial
<220>
<223> Elemento CDR3

<400> 49

Arg Arg Thr Trp His Ser Glu Leu
1 5

<210> 50
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 50

Gly Arg Ser Val Ser Arg Ser
1 5

<210> 51
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 51

Gly Arg Gly Ser Pro
1 5

<210> 52
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 52

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 53
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 53

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Met Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Gly Val Ser
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Ser Phe Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 54
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 54

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Asp Phe Ser Val Ser
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Val Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asp Ser Leu Ile Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Ser Phe Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 55
<211> 129
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 55

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Glu Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Leu Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 56
<211> 129
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 56
Gln Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Asp Tyr
20 25 30

Ser Gly Tyr Thr Tyr Thr Val Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Glu Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Asn Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Leu Met Asn Asn Leu Glu Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Glu Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 57
<211> 129

<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 57
Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Asp Tyr
20 25 30

Ser Gly Tyr Thr Tyr Thr Val Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Glu Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Asn Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Leu Met Asn Asn Leu Glu Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Glu Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 58
<211> 127
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 58

Ala Val Gln Leu Val Asp Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Ala His
20 25 30

Ser Val Tyr Thr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg
35 40 45

Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Ala Asn Thr Tyr Tyr Ala
50 55 60

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
65 70 75 80

Thr Val Asp Leu Leu Met Asn Cys Leu Lys Pro Glu Asp Thr Ala Val
85 90 95

Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Glu
100 105 110

Ala Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120 125

<210> 59
<211> 123
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 59

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ala Ala Ser Gly Arg Ser Phe Thr Gly Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Gln Leu Leu
35 40 45

Ala Ser Ile Ser Trp Arg Gly Asp Asn Thr Tyr Tyr Lys Glu Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Asn
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 60
<211> 123
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 60

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 61
<211> 117
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 61

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Gly Gly Gly
1 5 10 15

Ser Leu Arg Leu Ala Cys Ala Ala Ser Glu Arg Ile Phe Asp Leu Asn
20 25 30

Leu Met Gly Trp Tyr Arg Gln Gly Pro Gly Asn Glu Arg Glu Leu Val
35 40 45

Ala Thr Cys Ile Thr Val Gly Asp Ser Thr Asn Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Met Asp Tyr Thr Lys Gln Thr Val Tyr
65 70 75 80

Leu His Met Asn Ser Leu Arg Pro Glu Asp Thr Gly Leu Tyr Tyr Cys
85 90 95

Lys Ile Arg Arg Thr Trp His Ser Glu Leu Trp Gly Gln Gly Thr Gln
100 105 110

Val Thr Val Ser Ser
115

<210> 62
<211> 116
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 62

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Glu Gly Gly
1 5 10 15

Ser Leu Arg Leu Ala Cys Ala Ala Ser Glu Arg Ile Trp Asp Ile Asn
20 25 30

Leu Leu Gly Trp Tyr Arg Gln Gly Pro Gly Asn Glu Arg Glu Leu Val
35 40 45

Ala Thr Ile Thr Val Gly Asp Ser Thr Ser Tyr Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Tyr Asp Lys Asn Thr Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Gly Leu Tyr Tyr Cys Lys
85 90 95

Ile Arg Arg Thr Trp His Ser Glu Leu Trp Gly Gln Gly Thr Gln Val
100 105 110

Thr Val Ser Ser
115

<210> 63
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 63

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 64
<211> 116
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 64

Ala Val Gln Leu Val Asp Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Phe Gly Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Tyr Pro Gly Lys Glu Pro Glu Trp Val
35 40 45

Ser Ser Ile Asn Gly Arg Gly Asp Asp Thr Arg Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Ser Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Glu Tyr Tyr Cys
85 90 95

Thr Ile Gly Arg Ser Val Ser Arg Ser Arg Thr Gln Gly Thr Gln Val
100 105 110

Thr Val Ser Ser
115

<210> 65
<211> 114
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 65

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Thr Cys Thr Ala Ser Gly Phe Thr Phe Arg Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Asp Gln Glu Trp Val
35 40 45

Ser Ala Ile Ser Ala Asp Ser Ser Thr Lys Asn Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Lys Met Leu Tyr
65 70 75 80

Leu Glu Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Val Ile Gly Arg Gly Ser Pro Ser Ser Pro Gly Thr Gln Val Thr Val
100 105 110

Ser Ser

<210> 66
<211> 114
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 66

Gln Val Gln Leu Ala Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Thr Cys Thr Ala Ser Gly Phe Thr Phe Gly Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Glu Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Ala Asp Ser Ser Asp Lys Arg Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Lys Met Leu Tyr
65 70 75 80

Leu Glu Met Asn Ser Leu Lys Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Val Ile Gly Arg Gly Ser Pro Ala Ser Gln Gly Thr Gln Val Thr Val
100 105 110

Ser Ser

<210> 67
<211> 123
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 67
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30

Trp Met Tyr Trp Val Arg Val Ala Pro Gly Lys Gly Leu Glu Arg Ile
35 40 45

Ser Arg Asp Ile Ser Thr Gly Gly Gly Tyr Ser Tyr Tyr Ala Asp Ser
50 55 60

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu
65 70 75 80

Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr
85 90 95

Cys Ala Lys Asp Arg Glu Ala Gln Val Asp Thr Leu Asp Phe Asp Tyr
100 105 110

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 68
<211> 9
<212> PRT
<213> Artificial

<220>
<223> enlazador

<400> 68

Gly Gly Gly Gly Ser Gly Gly Gly Ser
1 5

<210> 69
<211> 30
<212> PRT
<213> Artificial

<220>
<223> enlazador

<400> 69

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser G ly
1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
20 25 30

<210> 70
<211> 239
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 70

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Gln Val Gln Leu
115 120 125

Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu
130 135 140

Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr Trp Met Tyr Trp
145 150 155 160

Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Glu Ile Asn
165 170 175

Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val Lys Gly Arg Phe
180 185 190

Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met Asn
195 200 205

Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg Ser Pro
210 215 220

Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
225 230 235

<210> 71
<211> 267
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 71

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Glu Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Leu Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Gln Val Gln Leu Val Glu
130 135 140

Ser Gly Gly Gly Leu Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys
145 150 155 160

Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro Ser Gly Tyr Thr Tyr Thr
165 170 175

Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
180 185 190

Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr Tyr Ala Asp Ser Val Lys
195 200 205

Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala Lys Asn Thr Val Asp Leu
210 215 220

Leu Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala
225 230 235 240

Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Gly Ser Tyr Asn Tyr
245 250 255

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
260 265

<210> 72
<211> 255
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 72

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Le u Val
130 135 140

Gln Ala Gly Gly Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser
145 150 155 160

Leu Ser Asn Tyr Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu
165 170 175

Arg Glu Leu Leu Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr
180 185 190

Lys Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys
195 200 205

Asn Thr Ile Tyr Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala
210 215 220

Val Tyr Tyr Cys Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly
225 230 235 240

Trp Asn Thr Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
245 250 255

<210> 73
<211> 260
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 73
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
145 150 155 160

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp
165 170 175

Tyr Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
180 185 190

Val Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser
195 200 205
Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu
210 215 220

Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr
225 230 235 240

Cys Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val
245 250 255

Thr Val Ser Ser
260

<210> 74
<211> 288
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 74

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Glu Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Leu Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
130 135 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser G In
145 150 155 160

Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly Ser
165 170 175

Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro Ser
180 185 190

Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu
195 200 205

Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr Tyr
210 215 220

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala Lys
225 230 235 240

Asn Thr Val Asp Leu Leu Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
245 250 255

Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser Val
260 265 270

Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
275 280 285

<210> 75
<211> 276
<212> PRT

<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 75

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser G ly
130 135 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln Leu V al Glu Ser
145 150 155 160

Gly Gly Gly Leu Val Gln Ala Gly Gly Ser L eu Ser Leu Ser Cys Ser
165 170 175

Ala Ser Gly Arg Ser Leu Ser Asn Tyr Tyr Met Gly Trp Phe Arg Gln
180 185 190

Ala Pro Gly Lys Glu Arg Glu Leu Leu Gly Asn Ile Ser Trp Arg Gly
195 200 205

Tyr Asn Ile Tyr Tyr Lys Asp Ser Val Lys Gly Arg Phe Thr Ile Ser
210 215 220

Arg Asp Asp Ala Lys Asn Thr Ile Tyr Leu Gln Met Asn Arg Leu Lys
225 230 235 240

Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala Ser Ile Leu Pro Leu
245 250 255

Ser Asp Asp Pro Gly Trp Asn Thr Tyr Trp Gly Gln Gly Thr Gln Val
260 265 270

Thr Val Ser Ser
275

<210> 76
<211> 115
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 76

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 77
<211> 115
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 77

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 78
<211> 129
<212> PRT
<213> Artificial

<220>

<223> constructo de nanocuerpo

<400> 78

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 79
<211> 129
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 79

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 80
<211> 129
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 80

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 81
<211> 129
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 81

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 82
<211> 129
<212> PRT
<213> Artificial
<220>
<223> constructo de nanocuerpo

<400> 82

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
115 120 125

Ser

<210> 83
<211> 123
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 83
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 84
<211> 123
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 84

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 85
<211> 123
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 85

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 86
<211> 123
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 86

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 87
<211> 115
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 87

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 88
<211> 115
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 88

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 89
<211> 115
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo
<400> 89

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 90
<211> 363
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 90

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30
Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln Leu
115 120 125

Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn Ser Leu Arg Leu
130 135 140

Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe Gly Met Ser Trp
145 150 155 160

Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val Ser Ser Ile Ser
165 170 175

Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val Lys Gly Arg Phe
180 185 190

Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr Leu Gln Met Asn
195 200 205

Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Thr Ile Gly Gly
210 215 220

Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr Val Ser Ser Gly
225 230 235 240
Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly
245 250 255

Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala
260 265 270

Ser Gly Phe Thr Phe Ser Asp Tyr Trp Met Tyr Trp Val Arg Gln Ala
275 280 285

Pro Gly Lys Gly Leu Glu Trp Val Ser Glu Ile Asn Thr Asn Gly Leu
290 295 300

Ile Thr Lys Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg
305 310 315 320

Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys Pro
325 330 335

Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg Ser Pro Ser Gly Phe Asn
340 345 350

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
355 360

<210> 91
<211> 391
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 91

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Glu Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Leu Met Asn Ser Leu Lys Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln Leu Val Glu
130 135 140

Ser Gly Gly Gly Leu Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys
145 150 155 160

Ala Ala Ser Gly Arg Thr Phe Ser Glu Pro Ser Gly Tyr Thr Tyr Thr
165 170 175

Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
180 185 190

Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr Tyr Ala Asp Ser Val Lys
195 200 205

Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala Lys Asn Thr Val Asp Leu
210 215 220

Leu Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala
225 230 235 240

Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Gly Ser Tyr Asn Tyr
245 250 255

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser
260 265 270

Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val
275 280 285

Gln Pro Gly Asn Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
290 295 300

Phe Arg Ser Phe Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Glu
305 310 315 320

Pro Glu Trp Val Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr
325 330 335

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
340 345 350

Thr Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
355 360 365

Val Tyr Tyr Cys Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly
370 375 380

Thr Gln Val Thr Val Ser Ser
385 390

<210> 92
<211> 379
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 92

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val
130 135 140

Gln Pro Gly Asn Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
145 150 155 160

Phe Arg Ser Phe Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Glu
165 170 175

Pro Glu Trp Val Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr
180 185 190

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
195 200 205

Thr Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala
210 215 220

1285

Val Tyr Tyr Cys Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly
225 230 235 240

Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser
245 250 255

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
260 265 270

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser Asn Tyr
275 280 285

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu
290 295 300

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
305 310 315 320

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
325 330 335

Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
340 345 350

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
355 360 365

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
370 375

<210> 93
<211> 384
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 93

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
145 150 155 160

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp
165 170 175

Tyr Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
180 185 190

Val Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser
195 200 205

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu
210 215 220

Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr
225 230 235 240

Cys Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Gln Val
245 250 255

Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln
260 265 270

Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn Ser Leu Arg
275 280 285

Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe Gly Met Ser
290 295 300

Trp Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val Ser Ser Ile
305 310 315 320

Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val Lys Gly Arg
325 330 335

Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr Leu Gln Met
340 345 350

Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Thr Ile Gly
355 360 365
Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr Val Ser Ser
370 375 380

<210> 94
<211> 400
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 94

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser G ly
130 135 140

Gly Gly Ser Ser Gly Gly Gly Gly Ser Glu Val Gln Val Val Glu Ser
145 150 155 160

Gly Gly Gly Leu Val Gln Ala Gly Gly Ser L eu Ser Leu Ser Cys Ser
165 170 175

Ala Ser Gly Arg Ser Leu Ser Asn Tyr Tyr Met Gly Trp Phe Arg Gln
180 185 190

Ala Pro Gly Lys Glu Arg Glu Leu Leu Gly Asn Ile Ser Trp Arg Gly
195 200 205

Tyr Asn Ile Tyr Tyr Lys Asp Ser Val Lys Gly Arg Phe Thr Ile Ser
210 215 220

Arg Asp Asp Ala Lys Asn Thr Ile Tyr Leu Gln Met Asn Arg Leu Lys
225 230 235 240

Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala Ser Ile Leu Pro Leu
245 250 255

Ser Asp Asp Pro Gly Trp Asn Thr Tyr Trp Gly Gln Gly Thr Gln Val
260 265 270

Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln
275 280 285

Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn Ser Leu Arg
290 295 300

Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe Gly Met Ser
305 310 315 320

Trp Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val Ser Ser Ile
325 330 335

Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val Lys Gly Arg
340 345 350
Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr Leu Gln Met
355 360 365

Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Thr Ile Gly
370 375 380

Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Gln Val Thr Val Ser Ser
385 390 395 400

<210> 95
<211> 115
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 95

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 96
<211> 115
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 96

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 97
<211> 129
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 97

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 98
<211> 129
<212> PRT
<213> Artificial

<220>

<223> constructo de nanocuerpo

<400> 98

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Glu Pro
20 25 30

Ser Gly Tyr Thr Tyr Thr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys
35 40 45

Gly Arg Glu Phe Val Ala Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr
50 55 60

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Ile Ala
65 70 75 80

Lys Asn Thr Val Asp Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Ala Arg Asp Gly Ile Pro Thr Ser Arg Ser
100 105 110

Val Gly Ser Tyr Asn Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
115 120 125

Ser

<210> 99
<211> 123
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 99

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 100
<211> 115
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 100

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 101
<211> 115
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 101

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 102
<211> 115
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 102

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 103
<211> 115
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 103

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 104
<211> 115
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo
<400> 104

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ile Gly Gly Ser Leu Ser Arg Ser Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 105
<211> 130
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 105

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gln Ile Ile Phe Gly Ser His
20 25 30
Val Ala Ala Trp Phe Arg Gln Ala Pro Gly Arg Glu Arg Glu Phe Val
35 40 45

Ala Glu Ile Arg Pro Ser Gly Asp Phe Gly Pro Glu Gly Glu Phe Glu
50 55 60

His Val Thr Ala Ser Leu Lys Gly Arg Phe Thr Ile Ala Lys Asn Ser
65 70 75 80

Val Asp Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp
85 90 95

Thr Ala Val Tyr Tyr Cys Ala Ala Ala Pro Tyr Arg Gly Gly Arg Asp
100 105 110

Tyr Arg Trp Glu Tyr Glu Tyr Glu Tyr Trp Gly Gln Gly Thr Gln Val
115 120 125

Thr Val
130

<210> 106
<211> 122
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 106

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Asn Ala Gly Ser Thr Ser Asn Ala Tyr
20 25 30

Ala Thr Gly Trp Phe Arg Arg Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Gly Ile Gln Trp Ser Gly Gly Asp Ala Phe Tyr Arg Asn Ser Val
50 55 60

Lys Gly Arg Phe Arg Ile Thr Arg Asp Pro Asp Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Asp Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr Cys Ala
85 90 95

Gln Lys Leu Ser Pro Tyr Tyr Asn Asp Phe Asp Ser Ser Asn Tyr Glu
100 105 110

Tyr Trp Gly Gln Gly Thr Gln Val Thr Val
115 120

<210> 107
<211> 119
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 107

Glu Val Gln Leu Val Glu Ser Gly Gly Asp Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Gln Leu Phe Ser Thr Asn
20 25 30

Asp Val Gly Trp Tyr Arg Arg Ala Pro Gly Lys Gln Arg Glu Leu Val
35 40 45

Ala Thr Ile Thr Asp Asp Gly Thr Thr Asp Tyr Gly Asp Asp Val Lys
50 55 60

Gly Arg Phe Val Ile Ser Arg Glu Gly Glu Met Val Tyr Leu Glu Met
65 70 75 80

Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Asn Ile Asn
85 90 95

Arg Leu Arg Ser Thr Trp Gly Ile Arg Tyr Asp Val Trp Gly Gln Gly
100 105 110

Thr Gln Val Thr Val Ser Ser
115

<210> 108
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 108

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Val Ser Gly Phe Thr Phe Ser Thr Thr
20 25 30

Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Phe Glu Glu Trp Val
35 40 45

Ser Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Gly Arg Arg Gly Tyr Gly Arg Asp Arg Ser Lys Gly Ile Gln Val Thr
100 105 110

Val Ala Ser
115

<210> 109
<211> 121
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 109

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Thr Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Phe Ser Ser Val
20 25 30

Ala Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gln Arg Glu Phe Leu
35 40 45

Ala Gly Val Gly Tyr Asp Gly Ser Ser Ile Arg Tyr Ala Glu Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ala Arg Gly Asn Arg Glu Ser Thr Val Phe
65 70 75 80

Leu Gln Met Glu Asn Leu Lys Pro Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95

Thr Ala Glu Pro Ile Gly Ala Tyr Glu Gly Leu Trp Thr Tyr Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 110
<211> 124
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<220>
<221> CARACTERÍSTICAS_MISCELÁNEAS
<222> (1)..(4)
<223> resiudos de aminoácidos desconocidos, probablemente EVQL, QVQL o una secuencia similar

<400> 110

Xaa Xaa Xaa Xaa Val Glu Ser Gly Gly Gly Leu Met Gln Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Met Phe Ser Asp Ser
20 25 30

Ala Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Thr Ile Ser Trp Asn Gly Gly Ser Ser Ser Tyr Ala Asp Phe Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Gly Leu Thr Pro Gln Asp Thr Ala Ile Tyr Tyr Cys
85 90 95

Ala Gly Ser Tyr Ser Asn Gly Asn Pro His Arg Phe Ser Gln Tyr Gln
100 105 110

Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 111

<211> 120
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<220>
<221> CARACTERÍSTICAS_MISCELÁNEAS
<222> (77)..(77)
<223> Residuo de aminoácido desconocido

<400> 111

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Gly Thr Tyr
20 25 30

Ala Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Ala Ile Ser Trp Gly Gly Gly Ser Ile Val Tyr Ala Glu Ser Ala
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Xaa Thr Met Tyr
65 70 75 80

Leu Gln Met Asp Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ala Asn Asn Ile Ala Thr Leu Arg Gln Gly Ser Trp Gly Gln
100 105 110

Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 112
<211> 127
<212> PRT
<213> Artificial
<220>
<223> nanocuerpo

<400> 112

Glu Val Gln Leu Val Glu Ser Gly Gly Glu Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Arg Asn Phe Val Thr Tyr
20 25 30

Ala Met Ser Trp Phe Arg Arg Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Ser Ile Ser Trp Ser Gly Asp Thr Thr Tyr Tyr Ser Asn Ser Val
50 55 60

Lys Gly Arg Phe Thr Val Ser Arg Asp Asn Gly Lys Asn Thr Ala Tyr
65 70 75 80

Leu Arg Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Asp Tyr Tyr Cys
85 90 95

Ala Val Val Gln Val Ile Asp Pro Ser Trp Ser Gly Val Asn Leu Asp
100 105 110

Asp Tyr Asp Tyr Leu Gly Ser Gly Thr Gln Val Thr Val Ser Ser
115 120 125

<210> 113
<211> 124
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 113

Glu Val Gln Leu Val Glu Ser Gly Gly Arg Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Lys Asn Ala Gly Ser Thr Ser Asn Ala Tyr
20 25 30

Ala Thr Gly Trp Phe Arg Arg Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Gly Ile Gln Trp Ser Gly Gly Asp Ala Phe Tyr Arg Asn Ser Val
50 55 60

Lys Gly Arg Phe Arg Ile Thr Arg Asp Pro Asp Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Asp Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr Cys Ala
85 90 95

Gln Lys Leu Ser Pro Tyr Tyr Asn Asp Phe Asp Ser Ser Asn Tyr Glu
100 105 110

Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 114
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 114

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Ala Thr Ile Ser Ser Ile Val
20 25 30

Met Leu Gly Trp Tyr Arg Gln Ala Pro Gly Lys Gln Arg Glu Trp Val
35 40 45

Ala Ser Ile Thr Ile Gly Ser Arg Thr Asn Tyr Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Phe Cys Asn
85 90 95

Ala Val Pro Pro Arg Asp Asp Tyr Trp Gly Gln Gly Thr Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 115
<211> 121
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 115

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Gln Thr Ser Ser Ser Tyr
20 25 30

Asp Met Gly Trp Phe Arg Gln Ala Pro Gly Glu Gly Arg Glu Phe Val
35 40 45

Ala Arg Ile Ser Gly Ser Asp Gly Ser Thr Tyr Tyr Ser Asp Arg Ala
50 55 60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Thr Lys Asn Met Val Tyr
65 70 75 80

Leu Gln Met Asp Arg Leu Lys Pro Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Arg Val Pro Arg Tyr Glu Asn Gln Trp Ser Ser Tyr Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 116
<211> 127
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 116

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ser Thr Phe Ser Thr Tyr
20 25 30

Asp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Asp Ser Gly Gly Gly Ser P ro Met Tyr Val Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Val Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Phe Ser Thr Gly Ala Asp Gly Gly Ser Trp Tyr Trp Ser Tyr
100 105 110

Gly Met Asp Ser Trp Gly Lys Gly Thr Gln Val Thr Val Ser Ser
115 120 125

<210> 117
<211> 121
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 117

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Glu Ala Ser Glu Arg Ser Ser Asn Arg Tyr
20 25 30

Asn Met Ala Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Leu
35 40 45

Ala Arg Val Asp Val Ser Gly Gly Asn Thr Leu Tyr Gly Asp Ser Val
50 55 60

Lys Asp Arg Phe Thr Val Ser Arg Ile Asn Gly Lys Asn Ala Met Tyr
65 70 75 80

Leu Gln Met Asn Asn Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr Cys
85 90 95

Ala Ala Gly Gly Trp Gly Thr Thr Gln Tyr Asp Tyr Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 118
<211> 117
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 118

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Cys Val Ser Ser Gly Cys Thr Phe Ser
20 25 30

Ala Tyr Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Ala Glu Glu
35 40 45

Trp Val Ser Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp
50 55 60

Ser Val Asn Gly Arg Phe Lys Ile Ser Arg Asp Asn Ala Lys Lys Thr
65 70 75 80

40
1308

Leu Tyr Leu Gln Met Asn Ser Leu Gly Pro Glu Asp Thr Ala Met Tyr
85 90 95

Tyr Cys Gln Arg Arg Gly Tyr Ala Leu Asp Arg Gly Gln Gly Thr Gln
100 105 110

Val Thr Val Ser Ser
115

<210> 119
<211> 129
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 119

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Thr Leu Ser Cys Ala Ser Ser Gly Arg Gly Phe Tyr Lys Asn
20 25 30

Ala Met Gly Trp Phe Arg Gln Pro Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Ser Ile Lys Trp Asn Gly Asn Asn Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Arg Gly Arg Phe Thr Ile Ser Arg Gly Asn Ala Lys Asn Thr Glu Asn
65 70 75 80

Thr Val Ser Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Asp
85 90 95

Tyr Tyr Cys Ala Ala Asp Ser Ser His Tyr Ser Tyr Val Tyr Ser Lys
100 105 110

Ala Tyr Glu Tyr Asp Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
115 120 125

Ser

<210> 120
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 120

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Phe Ser Gly Phe Ala Phe Ser Ala Ser
20 25 30

Ser Met Ala Trp Val Arg Gln Ala Pro Gly Lys Tyr Glu Glu Trp Val
35 40 45

Ser Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val
50 55 60

Gln Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Gly Arg Arg Gly Tyr Gly Arg Asp Arg Ser Gln Gly Ile Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 121
<211> 127
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<220>

<221> CARACTERÍSTICAS_MISCELÁNEAS
<222> (119)..(119)
<223> Residuo de aminoácido desconocido

<400> 121

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser Ser Tyr
20 25 30
Ala Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Ala Ile Ser Trp Ser Gly Thr Ile Thr Asn Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Gly Lys Asn Thr Val His
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr His Cys
85 90 95

Ala Val Val Gln Pro Tyr Ser Gly Gly Asp Tyr Tyr Thr Gly Val Glu
100 105 110

Glu Tyr Asp Tyr Trp Gly Xaa Gly Thr Gln Val Thr Val Ser Ser
115 120 125

<210> 122
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 122

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Val Ser Gly Phe Thr Phe Ser Ala Thr
20 25 30

Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Ala Glu Glu Trp Val
35 40 45

Ser Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asp Asp Leu Gln Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Gly Arg Arg Gly Tyr Gly Arg Asp Arg Ser Arg Gly Ile Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 123
<211> 121
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 123

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Gly Ala Phe Ser Asn Tyr
20 25 30

Asp Val Gly Trp Phe Arg Gln Ala Pro Gly Glu Gly Arg Glu Ile Val
35 40 45

Ala Arg Ile Ser Gly Ser Gly Asp Ser Thr Tyr Ser Ser Asn Arg Ala
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Arg Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Arg Ala Ala Arg Tyr Asn Gly Thr Trp Ser Ser Asn Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 124
<211> 117
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 124

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Glu Cys Val Ser Ser Gly Cys Thr Phe Ser
20 25 30

Ala Tyr Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Ala Glu Glu
35 40 45

Phe Val Ser Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asn
50 55 60

Ser Val Asn Gly Arg Phe Lys Ile Ser Arg Asp Asn Ala Lys Lys Thr
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Gly Pro Glu Asp Thr Ala Met Tyr
85 90 95

Tyr Cys Gln Arg Arg Gly Tyr Ala Leu Asp Arg Gly Gln Gly Thr Gln
100 105 110

Val Thr Val Ser Ser
115

<210> 125
<211> 121

<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 125

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Gln Thr Ser Ser Thr Ala
20 25 30

Asp Met Gly Trp Phe Arg Gln Pro Pro Gly Lys Gly Arg Glu Phe Val
35 40 45

Ala Arg Ile Ser Gly Ile Asp Gly Thr Thr Tyr Tyr Asp Glu Pro Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Lys Ala Gln Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asp Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Arg Ser Pro Arg Tyr Ala Asp Gln Trp Ser Ala Tyr Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 126
<211> 115
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo
<400> 126

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Val Ser Gly Phe Thr Phe Ser Thr Thr
20 25 30

Ser Met Thr Trp Val Arg Gln Ala Pro Gly Lys Phe Glu Glu Trp Val
35 40 45

Ser Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Gly Arg Arg Gly Tyr Gly Arg Asp Arg Ser Lys Gly Ile Gln Val Thr
100 105 110

Val Ser Ser
115

<210> 127
<211> 118
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 127

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Ala Ser Ala Ser Gly Val Lys Val Asn
20 25 30
Asp Met Gly Trp Tyr Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Val
35 40 45

Ala Thr Ile Thr Asp Asp Gly Arg Thr Asn Tyr Glu Asp Phe Ala Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Leu Pro Glu Asp Thr Ala Val Tyr Tyr Cys Asn
85 90 95

Ala Arg Thr Tyr Trp Ala His Leu Pro Thr Tyr Trp Gly Gln Gly Thr
100 105 110

Gln Val Thr Val Ser Ser
115

<210> 128
<211> 121
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 128

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Phe Gly Ser Val
20 25 30

Ala Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val
35 40 45

Ala Ala Ile Gly Tyr Asp Gly Asn Ser Ile Arg Tyr Gly Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ile Lys Asn Thr Met Tyr
65 70 75 80

Leu Glu Met Glu Asn Leu Asn Ala Asp Asp Thr Ala Arg Tyr Leu Cys
85 90 95

Ala Ala Glu Pro Leu Ala Arg Tyr Glu Gly Leu Trp Thr Tyr Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 129
<211> 121
<212> PRT
<213> Artificial

<220>
<223> nanocuerpo

<400> 129

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Ala
1 5 10 15

Ser Leu Arg Leu Ser Cys Thr Thr Ser Thr Arg Thr Asn Asp Arg Phe
20 25 30

Asn Met Ala Trp Phe His Gln Ala Pro Gly Lys Asp Arg Glu Phe Val
35 40 45

Ser Arg Ile Asp Val Ala Gly Tyr Asn Thr Ala Tyr Gly Asp Phe Val
50 55 60

Lys Gly Arg Phe Thr Val Ser Arg Asp Ser Ala Glu Asn Thr Val Val
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Tyr Cys
85 90 95

Ala Ala Gly Gly Trp Gly Ile Ser Gln Ser Asp Tyr Asp Leu Trp Gly
100 105 110

Gln Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> 130
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 130

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
20 25 30

<210> 131
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 131

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Met Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Gly
20 25 30

<210> 132
<211> 30
<212> PRT
<213> Artificial
<220>
<223> Elemento FR1

<400> 132

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Asp Phe Ser
20 25 30

<210> 133
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 133

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser
20 25 30

<210> 134
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 134

Gln Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser
20 25 30

<210> 135
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 135

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser
20 25 30

<210> 136
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 136

Ala Val Gln Leu Val Asp Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

1319

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser
20 25 30

<210> 137
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 137

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ala Ala Ser Gly Arg Ser Phe Thr
20 25 30

<210> 138
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 138

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Ser Leu Ser Cys Ser Ala Ser Gly Arg Ser Leu Ser
20 25 30

<210> 139
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 139

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gln Ile Ile Phe Gly
20 25 30

<210> 140
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 140

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Lys Asn Ala Gly Ser Thr Ser Asn
20 25 30

<210> 141
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 141

Glu Val Gln Leu Val Glu Ser Gly Gly Asp Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Gln Leu Phe Ser
20 25 30

<210> 142
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 142

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Val Ser Gly Phe Thr Phe Ser
20 25 30

<210> 143
<211> 30

<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 143
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Thr Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Phe Ser
20 25 30

<210> 144
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<220>
<221> CARACTERÍSTICAS_MISCELÁNEAS
<222> (1)..(4)
<223> Resiudos de aminoácidos desconocidos, probablemente EVQL, QVQL o una secuencia similar

<400> 144

Xaa Xaa Xaa Xaa Val Glu Ser Gly Gly Gly Leu Met Gln Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Met Phe Ser
20 25 30

<210> 145
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 145

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Gly
20 25 30

<210> 146
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 146

Glu Val Gln Leu Val Glu Ser Gly Gly Glu Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Arg Asn Phe Val
20 25 30

<210> 147
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 147

Glu Val Gln Leu Val Glu Ser Gly Gly Arg Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Asn Ala Gly Ser Thr Ser Asn
20 25 30

<210> 148
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 148

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Ala Thr Ile Ser Ser
20 25 30

<210> 149
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 149

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Gln Thr Ser Ser
20 25 30

<210> 150
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 150

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ser Thr Phe Ser
20 25 30

<210> 151
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 151

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Asp
1 5 10 15
Ser Leu Arg Leu Ser Cys Glu Ala Ser Glu Arg Ser Ser Asn
20 25 30

<210> 152
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 152

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Cys Val Ser Ser Gly Cys Thr
20 25 30

<210> 153
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 153

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Asp
1 5 10 15

Ser Leu Thr Leu Ser Cys Ala Ser Ser Gly Arg Gly Phe Tyr
20 25 30

<210> 154
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 154
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Phe Ser Gly Phe Ala Phe Ser
20 25 30

<210> 155
<211> 30
<212> PRT
<213> Artificial

<220>

<223> Elemento FR1

<400> 155

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Thr Phe Ser
20 25 30

<210> 156
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 156

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Val Ser Gly Phe Thr Phe Ser
20 25 30

<210> 157
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 157

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Gly Ala Phe Ser
20 25 30

<210> 158
<211> 30
<212> PRT
<213> Artificial

<220>

<223> Elemento FR1

<400> 158

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Glu Cys Val Ser Ser Gly Cys Thr
20 25 30

<210> 159
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 159

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Gln Thr Ser Ser
20 25 30

<210> 160
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 160

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Val Ser Gly Phe Thr Phe Ser
20 25 30

<210> 161
<211> 30
<212> PRT
<213> Artificial

<220>

<223> Elemento FR1

<400> 161

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Ala Ser Ala Ser Gly Val Lys
20 25 30

<210> 162
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 162

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Phe Gly
20 25 30

<210> 163
<211> 29
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 163

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Ala Gly Ala
1 5 10 15

Ser Leu Arg Leu Ser Cys Thr Thr Ser Thr Arg Thr Asn
20 25

<210> 164
<211> 5
<212> PRT
<213> Artificial

<220>

<223> Elemento CDR1

<400> 164

Asp Tyr Trp Met Tyr
1 5

<210> 165
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 165

Val Ser Trp Met Tyr
1 5

<210> 166
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 166

Val Ser Trp Met Tyr
1 5

<210> 167
<211> 10
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 167

Glu Pro Ser Gly Tyr Thr Tyr Thr Ile Gly
1 5 10

<210> 168
<211> 10
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 168

Asp Tyr Ser Gly Tyr Thr Tyr Thr Val Gly
1 5 10

<210> 169
<211> 10
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 169

Asp Tyr Ser Gly Tyr Thr Tyr Thr Val Gly
1 5 10

<210> 170
<211> 8
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 170

Ala His Ser Val Tyr Thr Met Gly
1 5

<210> 171
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 171

Gly Tyr Tyr Met Gly
1 5

<210> 172
<211> 5
<212> PRT

<213> Artificial

<220>
<223> Elemento CDR1

<400> 172

Asn Tyr Tyr Met Gly
1 5

<210> 173
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 173

Ser His Val Ala Ala
1 5

<210> 174
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 174

Ala Tyr Ala Thr Gly
1 5

<210> 175
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 175

Thr Asn Asp Val Gly
1 5

<210> 176

<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 176

Thr Thr Ser Met Thr
1 5

<210> 177
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 177

Ser Val Ala Met Gly
1 5

<210> 178
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 178

Asp Ser Ala Met Gly
1 5

<210> 179
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 179

Thr Tyr Ala Met Gly
1 5

<210> 180
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 180

Thr Tyr Ala Met Ser
1 5

<210> 181
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 181

Ala Tyr Ala Thr Gly
1 5

<210> 182
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 182

Ile Val Met Leu Gly
1 5

<210> 183
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 183

Ser Tyr Asp Met Gly
1 5

<210> 184
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 184

Thr Tyr Asp Met Ser
1 5

<210> 185
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 185

Arg Tyr Asn Met Ala
1 5

<210> 186
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 186

Phe Ser Ala Tyr Ser Met Thr
1 5

<210> 187
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 187

Lys Asn Ala Met Gly
1 5

<210> 188
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 188

Ala Ser Ser Met Ala
1 5

<210> 189
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 189

Ser Tyr Ala Met Gly
1 5

<210> 190
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 190

Ala Thr Ser Met Thr
1 5

<210> 191
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 191

Asn Tyr Asp Val Gly
1 5

<210> 192
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 192

Phe Ser Ala Tyr Ser Met Thr
1 5

<210> 193
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 193

Thr Ala Asp Met Gly
1 5

<210> 194
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 194

Thr Thr Ser Met Thr
1 5

<210> 195
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

1336

<400> 195

Val Asn Asp Met Gly
1 5

<210> 196
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 196

Ser Val Ala Met Gly
1 5

<210> 197
<211> 6
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 197

Asp Arg Phe Asn Met Ala
1 5

<210> 198
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 198

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
1 5 10

<210> 199
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

1337

<400> 199

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
1 5 10

<210> 200
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 200

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
1 5 10

<210> 201
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 201

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 202
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 202

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 203
<211> 14
<212> PRT
<213> Artificial

<220>

<223> Elemento FR2

<400> 203

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 204
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 204

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 205
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 205

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Gln Leu Leu Ala
1 5 10

<210> 206
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 206

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Leu Gly
1 5 10

<210> 207
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 207

Trp Phe Arg Gln Ala Pro Gly Arg Glu Arg Glu Phe Val Ala
1 5 10

<210> 208
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 208

Trp Phe Arg Arg Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 209
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 209

Trp Tyr Arg Arg Ala Pro Gly Lys Gln Arg Glu Leu Val Ala
1 5 10

<210> 210
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 210

Trp Val Arg Gln Ala Pro Gly Lys Phe Glu Glu Trp Val Ser
1 5 10

<210> 211
<211> 14
<212> PRT

<213> Artificial

<220>
<223> Elemento FR2

<400> 211

Trp Phe Arg Gln Ala Pro Gly Lys Gln Arg Glu Phe Leu Ala
1 5 10

<210> 212
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 212

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 213
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 213

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 214
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 214

Trp Phe Arg Arg Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 215

<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 215

Trp Phe Arg Arg Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 216
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 216

Trp Tyr Arg Gln Ala Pro Gly Lys Gln Arg Glu Trp Val Ala
1 5 10

<210> 217
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 217

Trp Phe Arg Gln Ala Pro Gly Glu Gly Arg Glu Phe Val Ala
1 5 10

<210> 218
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 218

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
1 5 10

<210> 219
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 219

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Leu Ala
1 5 10

<210> 220
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 220

Trp Val Arg Gln Ala Pro Gly Lys Ala Glu Glu Trp Val Ser
1 5 10

<210> 221
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 221

Trp Phe Arg Gln Pro Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 222
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 222

Trp Val Arg Gln Ala Pro Gly Lys Tyr Glu Glu Trp Val Ser
1 5 10

<210> 223
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 223

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 224
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 224

Trp Val Arg Gln Ala Pro Gly Lys Ala Glu Glu Trp Val Ser
1 5 10

<210> 225
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 225

Trp Phe Arg Gln Ala Pro Gly Glu Gly Arg Glu Ile Val Ala
1 5 10

<210> 226
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 226

Trp Val Arg Gln Ala Pro Gly Lys Ala Glu Glu Phe Val Ser
1 5 10

<210> 227
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 227

Trp Phe Arg Gln Pro Pro Gly Lys Gly Arg Glu Phe Val Ala
1 5 10

<210> 228
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 228

Trp Val Arg Gln Ala Pro Gly Lys Phe Glu Glu Trp Val Ser
1 5 10

<210> 229
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 229

Trp Tyr Arg Gln Ala Pro Gly Lys Glu Arg Glu Leu Val Ala
1 5 10

<210> 230
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 230

Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ala
1 5 10

<210> 231
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 231

Trp Phe His Gln Ala Pro Gly Lys Asp Arg Glu Phe Val Ser
1 5 10

<210> 232
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 232

Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val Lys
1 5 10 15

Gly

<210> 233
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 233

Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val Lys
1 5 10 15

Gly

<210> 234
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 234

Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Val Asp Ser Val Lys
1 5 10 15

Gly

<210> 235
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 235

Arg Ile Tyr Trp Ser Ser Gly Leu Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 236
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 236

Arg Ile Tyr Trp Ser Ser Gly Asn Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 237
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 237

Arg Ile Tyr Trp Ser Ser Gly Asn Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 238
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 238

Arg Ile Tyr Trp Ser Ser Ala Asn Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 239
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 239

Ser Ile Ser Trp Arg Gly Asp Asn Thr Tyr Tyr Lys Glu Ser Val Lys
1 5 10 15

Gly

<210> 240
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 240

Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val Lys
1 5 10 15

Gly

<210> 241
<211> 23
<212> PRT

<213> Artificial

<220>
<223> Elemento CDR2

<400> 241

Glu Ile Arg Pro Ser Gly Asp Phe Gly Pro Glu Gly Glu Phe Glu His
1 5 10 15

Val Thr Ala Ser Leu Lys Gly
20

<210> 242
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 242

Gly Ile Gln Trp Ser Gly Gly Asp Ala Phe Tyr Arg Asn Ser Val Lys
1 5 10 15

Gly

<210> 243
<211> 16
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 243

Thr Ile Thr Asp Asp Gly Thr Thr Asp Tyr Gly Asp Asp Val Lys Gly
1 5 10 15

<210> 244
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 244

Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 245
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 245

Gly Val Gly Tyr Asp Gly Ser Ser Ile Arg Tyr Ala Glu Ser Val Lys
1 5 10 15

Gly

<210> 246
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 246

Thr Ile Ser Trp Asn Gly Gly Ser Ser Ser Tyr Ala Asp Phe Val Lys
1 5 10 15

Gly

<210> 247
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 247

Ala Ile Ser Trp Gly Gly Gly Ser Ile Val Tyr Ala Glu Ser Ala Lys
1 5 10 15

Gly

<210> 248
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 248

Ser Ile Ser Trp Ser Gly Asp Thr Thr Tyr Tyr Ser Asn Ser Val Lys
1 5 10 15

Gly

<210> 249
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 249

Gly Ile Gln Trp Ser Gly Gly Asp Ala Phe Tyr Arg Asn Ser Val Lys
1 5 10 15

Gly

<210> 250
<211> 16
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 250

Ser Ile Thr Ile Gly Ser Arg Thr Asn Tyr Ala Asp Ser Val Lys Gly
1 5 10 15

<210> 251
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 251

Arg Ile Ser Gly Ser Asp Gly Ser Thr Tyr Tyr Ser Asp Arg Ala Lys
1 5 10 15

Asp

<210> 252
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 252

Gly Ile Asp Ser Gly Gly Gly Ser Pro Met Tyr Val Asp Ser Val Lys
1 5 10 15

Gly

<210> 253
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 253

Arg Val Asp Val Ser Gly Gly Asn Thr Leu Tyr Gly Asp Ser Val Lys
1 5 10 15

Asp

<210> 254
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 254

Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val Asn
1 5 10 15

Gly

<210> 255
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 255

Ser Ile Lys Trp Asn Gly Asn Asn Thr Tyr Tyr Ala Asp Ser Val Arg
1 5 10 15

Gly

<210> 256
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 256

Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val Gln
1 5 10 15

Gly

<210> 257
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 257

Ala Ile Ser Trp Ser Gly Thr Ile Thr Asn Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 258
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 258

Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 259
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 259

Arg Ile Ser Gly Ser Gly Asp Ser Thr Tyr Ser Ser Asn Arg Ala Lys
1 5 10 15

Gly

<210> 260
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 260

Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asn Ser Val Asn
1 5 10 15

Gly

<210> 261
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 261

Arg Ile Ser Gly Ile Asp Gly Thr Thr Tyr Tyr Asp Glu Pro Val Lys
1 5 10 15

Gly

<210> 262
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 262

Phe Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 263
<211> 16
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 263

Thr Ile Thr Asp Asp Gly Arg Thr Asn Tyr Glu Asp Phe Ala Lys Gly
1 5 10 15

<210> 264
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 264

Ala Ile Gly Tyr Asp Gly Asn Ser Ile Arg Tyr Gly Asp Ser Val Lys
1 5 10 15

Gly

<210> 265
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 265

Arg Ile Asp Val Ala Gly Tyr Asn Thr Ala Tyr Gly Asp Phe Val Lys
1 5 10 15

Gly

<210> 266
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 266

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg
20 25 30

<210> 267
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 267

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg
20 25 30

<210> 268
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 268

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asp Ser Leu Ile Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg
20 25 30

<210> 269
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 269

Arg Phe Thr Ile Ser Arg Asp Ile Ala Lys Asn Thr Val Asp Leu Leu
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 270
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 270

Arg Phe Thr Ile Ser Arg Asp Ile Ala Lys Asn Thr Val Asp Leu Leu
1 5 10 15

Met Asn Asn Leu Glu Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 271
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 271

Arg Phe Thr Ile Ser Arg Asp Ile Ala Lys Asn Thr Val Asp Leu Leu
1 5 10 15

Met Asn Asn Leu Glu Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 272
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 272

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Asp Leu Leu
1 5 10 15

Met Asn Cys Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 273
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 273

Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 274
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 274

Arg Phe Thr Ile Ser Arg Asp Asp Ala Lys Asn Thr Ile Tyr Leu Gln
1 5 10 15

Met Asn Arg Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 275
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 275

Arg Phe Thr Ile Ala Lys Asn Ser Val Asp Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 276
<211> 31
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 276

Arg Phe Arg Ile Thr Arg Asp Pro Asp Asn Thr Val Tyr Leu Gln Met
1 5 10 15

Asn Asp Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr Cys Ala Gln
20 25 30

<210> 277
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 277

Arg Phe Val Ile Ser Arg Glu Gly Glu Met Val Tyr Leu Glu Met Asn
1 5 10 15

Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Asn Ile
20 25 30

<210> 278
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 278

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Gly Arg
20 25 30

<210> 279
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 279

Arg Phe Thr Ile Ala Arg Gly Asn Arg Glu Ser Thr Val Phe Leu Gln
1 5 10 15

Met Glu Asn Leu Lys Pro Glu Asp Thr Ala Val Tyr Phe Cys Thr Ala
20 25 30

<210> 280
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 280

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Gly Leu Thr Pro Gln Asp Thr Ala Ile Tyr Tyr Cys Ala Gly
20 25 30

<210> 281
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<220>
<221> CARACTERÍSTICAS_MISCELÁNEAS
<222> (11)..(11)
<223> residuo de aminoácido desconocido

<400> 281

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Xaa Thr Met Tyr Leu Gln
1 5 10 15

Met Asp Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 282
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 282

Arg Phe Thr Val Ser Arg Asp Asn Gly Lys Asn Thr Ala Tyr Leu Arg
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Asp Tyr Tyr Cys Ala Val
20 25 30

<210> 283
<211> 31
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 283

Arg Phe Arg Ile Thr Arg Asp Pro Asp Asn Thr Val Tyr Leu Gln Met
1 5 10 15

Asn Asp Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr Cys Ala Gln
20 25 30

<210> 284
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 284

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Phe Cys Asn Ala
20 25 30

<210> 285
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 285

Arg Phe Thr Ile Ser Arg Asp Asn Thr Lys Asn Met Val Tyr Leu Gln
1 5 10 15

Met Asp Arg Leu Lys Pro Asp Asp Thr Ala Val Tyr Tyr Cys Arg Val
20 25 30

<210> 286
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 286

Arg Phe Thr Val Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys
20 25 30

<210> 287
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 287

Arg Phe Thr Val Ser Arg Ile Asn Gly Lys Asn Ala Met Tyr Leu Gln
1 5 10 15

Met Asn Asn Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr Cys Ala Ala
20 25 30

<210> 288
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 288

Arg Phe Lys Ile Ser Arg Asp Asn Ala Lys Lys Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Gly Pro Glu Asp Thr Ala Met Tyr Tyr Cys Gln Arg
20 25 30

<210> 289
<211> 35
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 289

Arg Phe Thr Ile Ser Arg Gly Asn Ala Lys Asn Thr Glu Asn Thr Val
1 5 10 15

Ser Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Asp Tyr Tyr
20 25 30

Cys Ala Ala
35

<210> 290
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 290

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys Gly Arg
20 25 30

<210> 291
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 291

Arg Phe Thr Ile Ser Arg Asp Asn Gly Lys Asn Thr Val His Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr His Cys Ala Val
20 25 30

<210> 292
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 292

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asp Asp Leu Gln Ser Glu Asp Thr Ala Met Tyr Tyr Cys Gly Arg
20 25 30

<210> 293
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 293

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Arg Glu Asp Thr Ala Val Tyr Tyr Cys Arg Ala
20 25 30

<210> 294
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 294

Arg Phe Lys Ile Ser Arg Asp Asn Ala Lys Lys Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Gly Pro Glu Asp Thr Ala Met Tyr Tyr Cys Gln Arg
20 25 30

<210> 295
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 295

Arg Phe Thr Ile Ser Arg Asp Lys Ala Gln Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asp Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Arg Ser
20 25 30

<210> 296
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 296

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Gly Arg
20 25 30

<210> 297
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 297

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Leu Pro Glu Asp Thr Ala Val Tyr Tyr Cys Asn Ala
20 25 30

<210> 298
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 298

Arg Phe Thr Ile Ser Arg Asp Asn Ile Lys Asn Thr Met Tyr Leu Glu
1 5 10 15

Met Glu Asn Leu Asn Ala Asp Asp Thr Ala Arg Tyr Leu Cys Ala Ala
20 25 30

<210> 299
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 299

Arg Phe Thr Val Ser Arg Asp Ser Ala Glu Asn Thr Val Val Leu Gln
1 5 10 15

Met Asn Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Tyr Cys Ala Ala
20 25 30

<210> 300
<211> 6
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 300

Ser Pro Ser Gly Phe Asn
1 5

<210> 301
<211> 6
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 301

Ser Pro Ser Gly Ser Phe
1 5

<210> 302
<211> 6
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 302

Ser Pro Ser Gly Ser Phe
1 5

<210> 303
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 303

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Gly Ser Tyr Asn Tyr
1 5 10 15

<210> 304
<211> 15
<212> PRT

<213> Artificial

<220>
<223> Elemento CDR3

<400> 304

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Glu Ser Tyr Asn Tyr
1 5 10 15

<210> 305
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 305

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Glu Ser Tyr Asn Tyr
1 5 10 15

<210> 306
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 306

Arg Asp Gly Ile Pro Thr Ser Arg Ser Val Glu Ala Tyr Asn Tyr
1 5 10 15

<210> 307
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 307

Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Asn
1 5 10

<210> 308

1358

<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 308

Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
1 5 10

<210> 309
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 309

Ala Pro Tyr Arg Gly Gly Arg Asp Tyr Arg Trp Glu Tyr Glu Tyr Glu
1 5 10 15

Tyr

<210> 310
<211> 16
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 310

Lys Leu Ser Pro Tyr Tyr Asn Asp Phe Asp Ser Ser Asn Tyr Glu Tyr
1 5 10 15

<210> 311
<211> 13
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 311

Asn Arg Leu Arg Ser Thr Trp Gly Ile Arg Tyr Asp Val
1 5 10

<210> 312
<211> 6
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 312

Arg Gly Tyr Gly Arg Asp
1 5

<210> 313
<211> 12
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 313

Glu Pro Ile Gly Ala Tyr Glu Gly Leu Trp Thr Tyr
1 5 10

<210> 314
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 314

Ser Tyr Ser Asn Gly Asn Pro His Arg Phe Ser Gln Tyr Gln Tyr
1 5 10 15

<210> 315
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

1370

<400> 315

Ala Asn Asn Ile Ala Thr Leu Arg Gln Gly Ser
1 5 10

<210> 316
<211> 18
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 316

Val Gln Val Ile Asp Pro Ser Trp Ser Gly Val Asn Leu Asp Asp Tyr
1 5 10 15

Asp Tyr

<210> 317
<211> 16
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 317

Lys Leu Ser Pro Tyr Tyr Asn Asp Phe Asp Ser Ser Asn Tyr Glu Tyr
1 5 10 15

<210> 318
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 318

Val Pro Pro Arg Asp Asp Tyr
1 5

<210> 319

<211> 12
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 319

Pro Arg Tyr Glu Asn Gln Trp Ser Ser Tyr Asp Tyr
1 5 10

<210> 320
<211> 18
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 320

Phe Ser Thr Gly Ala Asp Gly Gly Ser Trp Tyr Trp Ser Tyr Gly Met
1 5 10 15

Asp Ser

<210> 321
<211> 12
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 321

Gly Gly Trp Gly Thr Thr Gln Tyr Asp Tyr Asp Tyr
1 5 10

<210> 322
<211> 6
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 322

Arg Gly Tyr Ala Leu Asp
1 5

<210> 323
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 323

Asp Ser Ser His Tyr Ser Tyr Val Tyr Ser Lys Ala Tyr Glu Tyr Asp
1 5 10 15

Tyr

<210> 324
<211> 6
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 324

Arg Gly Tyr Gly Arg Asp
1 5

<210> 325
<211> 18
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 325

Val Gln Pro Tyr Ser Gly Gly Asp Tyr Tyr Thr Gly Val Glu Glu Tyr
1 5 10 15

Asp Tyr

<210> 326
<211> 6

<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 326

Arg Gly Tyr Gly Arg Asp
1 5

<210> 327
<211> 12
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 327

Ala Arg Tyr Asn Gly Thr Trp Ser Ser Asn Asp Tyr
1 5 10

<210> 328
<211> 6
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 328

Arg Gly Tyr Ala Leu Asp
1 5

<210> 329
<211> 12
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 329

Pro Arg Tyr Ala Asp Gln Trp Ser Ala Tyr Asp Tyr
1 5 10

<210> 330
<211> 6

<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 330

Arg Gly Tyr Gly Arg Asp
1 5

<210> 331
<211> 10
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 331

Arg Thr Tyr Trp Ala His Leu Pro Thr Tyr
1 5 10

<210> 332
<211> 12
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 332

Glu Pro Leu Ala Arg Tyr Glu Gly Leu Trp Thr Tyr
1 5 10

<210> 333
<211> 12
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 333

Gly Gly Trp Gly Ile Ser Gln Ser Asp Tyr Asp Leu
1 5 10

<210> 334
<211> 11

<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 334

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 335
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 335

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 336
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 336

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 337
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 337

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 338

<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 338

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 339
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 339

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 340
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 340

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 341
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 341

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 342
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 342

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 343
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 343

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 344
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 344

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 345
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 345

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 346
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 346

Arg Ser Lys Gly Ile Gln Val Thr Val Ala Ser
1 5 10

<210> 347
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 347

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 348
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 348

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 349
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 349

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 350
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 350

Leu Gly Ser Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 351
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 351

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 352
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 352

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 353
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 353

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 354
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 354

Trp Gly Lys Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 355
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 355

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 356
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 356

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 357
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 357

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 358
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 358

Arg Ser Gln Gly Ile Gln Val Thr Val Ser Ser
1 5 10

<210> 359
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<220>
<221> CARACTERÍSTICAS_MISCELÁNEAS
<222> (3)..(3)
<223> Residuo de aminoácido desconocido

<400> 359

Trp Gly Xaa Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 360
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 360

Arg Ser Arg Gly Ile Gln Val Thr Val Ser Ser
1 5 10

<210> 361
<211> 11
<212> PRT
<213> Artificial

<220>

<223> Elemento FR4

<400> 361

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 362
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 362

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 363
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 363

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 364
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 364

Arg Ser Lys Gly Ile Gln Val Thr Val Ser Ser
1 5 10

<210> 365
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 365

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 366
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 366

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 367
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 367

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 368
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 368

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Gly Gly Gly
1 5 10 15

Ser Leu Arg Leu Ala Cys Ala Ala Ser Glu Arg Ile Phe Asp
20 25 30

1384

<210> 369
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 369

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Glu Gly Gly
1 5 10 15

Ser Leu Arg Leu Ala Cys Ala Ala Ser Glu Arg Ile Trp Asp
20 25 30

<210> 370
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 370

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg
20 25 30

<210> 371
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 371

Ala Val Gln Leu Val Asp Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Phe Gly
20 25 30

1385

<210> 372
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 372

Ala Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Thr Cys Thr Ala Ser Gly Phe Thr Phe Arg
20 25 30

<210> 373
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 373

Gln Val Gln Leu Ala Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Thr Cys Thr Ala Ser Gly Phe Thr Phe Gly
20 25 30

<210> 374
<211> 30
<212> PRT
<213> Artificial

<220>
<223> Elemento FR1

<400> 374

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
20 25 30

11386

<210> 375
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 375

Leu Asn Leu Met Gly
1 5

<210> 376
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 376

Ile Asn Leu Leu Gly
1 5

<210> 377
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 377

Ser Phe Gly Met Ser
1 5

<210> 378
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 378

Ser Phe Gly Met Ser
1 5

<210> 379
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 379

Ser Phe Gly Met Ser
1 5

<210> 380
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 380

Ser Phe Gly Met Ser
1 5

<210> 381
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR1

<400> 381

Asn Tyr Trp Met Tyr
1 5

<210> 382
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 382

Trp Tyr Arg Gln Gly Pro Gly Asn Glu Arg Glu Leu Val Ala
1 5 10

<210> 383
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 383

Trp Tyr Arg Gln Gly Pro Gly Asn Glu Arg Glu Leu Val Ala
1 5 10

<210> 384
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 384

Trp Val Arg Gln Ala Pro Gly Lys Glu Pro Glu Trp Val Ser
1 5 10

<210> 385
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 385

Trp Val Arg Gln Tyr Pro Gly Lys Glu Pro Glu Trp Val Ser
1 5 10

<210> 386
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 386

Trp Val Arg Gln Ala Pro Gly Lys Asp Gln Glu Trp Val Ser
1 5 10

<210> 387
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 387

Trp Val Arg Gln Ala Pro Gly Glu Gly Leu Glu Trp Val Ser
1 5 10

<210> 388
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Elemento FR2

<400> 388

Trp Val Arg Val Ala Pro Gly Lys Gly Leu Glu Arg Ile Ser
1 5 10

<210> 389
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 389

Thr Cys Ile Thr Val Gly Asp Ser Thr Asn Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 390
<211> 16
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 390

Thr Ile Thr Val Gly Asp Ser Thr Ser Tyr Ala Asp Ser Val Lys Gly
1 5 10 15

<210> 391
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 391

Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 392
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 392

Ser Ile Asn Gly Arg Gly Asp Asp Thr Arg Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 393
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 393

Ala Ile Ser Ala Asp Ser Ser Thr Lys Asn Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 394
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 394

Ala Ile Ser Ala Asp Ser Ser Asp Lys Arg Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 395
<211> 18
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR2

<400> 395

Arg Asp Ile Ser Thr Gly Gly Gly Tyr Ser Tyr Tyr Ala Asp Ser Val
1 5 10 15

Lys Gly

<210> 396
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 396

Arg Phe Thr Ile Ser Met Asp Tyr Thr Lys Gln Thr Val Tyr Leu His
1 5 10 15

Met Asn Ser Leu Arg Pro Glu Asp Thr Gly Leu Tyr Tyr Cys Lys Ile
20 25 30

1392

<210> 397
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 397

Arg Phe Thr Ile Ser Arg Asp Tyr Asp Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Arg Pro Glu Asp Thr Gly Leu Tyr Tyr Cys Lys Ile
20 25 30

<210> 398
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 398

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Thr Ile
20 25 30

<210> 399
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 399

Arg Phe Ser Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Glu Tyr Tyr Cys Thr Ile
20 25 30

<210> 400
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 400

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Lys Met Leu Tyr Leu Glu
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Val Ile
20 25 30

<210> 401
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 401

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Lys Met Leu Tyr Leu Glu
1 5 10 15

Met Asn Ser Leu Lys Ser Glu Asp Thr Ala Val Tyr Tyr Cys Val Ile
20 25 30

<210> 402
<211> 32
<212> PRT
<213> Artificial

<220>
<223> Elemento FR3

<400> 402

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Lys
20 25 30

<210> 403
<211> 8
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 403

Arg Arg Thr Trp His Ser Glu Leu
1 5

<210> 404
<211> 8
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 404

Arg Arg Thr Trp His Ser Glu Leu
1 5

<210> 405
<211> 6
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 405

Gly Gly Ser Leu Ser Arg
1 5

<210> 406
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 406

Gly Arg Ser Val Ser Arg Ser
1 5

<210> 407
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 407

Gly Arg Gly Ser Pro
1 5

<210> 408
<211> 5
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 408

Gly Arg Gly Ser Pro
1 5

<210> 409
<211> 13
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 409

Asp Arg Glu Ala Gln Val Asp Thr Leu Asp Phe Asp Tyr
1 5 10

<210> 410
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 410

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 411
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 411

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 412
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 412

Ser Ser Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 413
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 413

Arg Thr Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 414
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 414

Ser Ser Pro Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 415
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 415

Ala Ser Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 416
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Elemento FR4

<400> 416

Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser
1 5 10

<210> 417
<211> 363
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 417

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln Leu
115 120 125

Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Asn Ser Leu Arg Leu
130 135 140

Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe Gly Met Ser Trp
145 150 155 160

Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ser Ile Ser
165 170 175

Gly Ser Gly Ser Asp Thr Leu Tyr Ala Asp Ser Val Lys Gly Arg Phe
180 185 190

Thr Ile Ser Arg Asp Asn Ala Lys Thr Thr Leu Tyr Leu Gln Met Asn
195 200 205

Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys Thr Ile Gly Gly
210 215 220

Ser Leu Ser Arg Ser Ser Gln Gly Thr Leu Val Thr Val Ser Ser Gly
225 230 235 240

Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly
245 250 255

Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala
260 265 270

Ser Gly Phe Thr Phe Ser Asp Tyr Trp Met Tyr Trp Val Arg Gln Ala
275 280 285

Pro Gly Lys Gly Leu Glu Trp Val Ser Glu Ile Asn Thr Asn Gly Leu
290 295 300

Ile Thr Lys Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg
305 310 315 320

Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Pro
325 330 335

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Pro Ser Gly Phe Asn
340 345 350

Arg Gly Gln Gly Thr Leu Val Thr Val Ser Ser
355 360

<210> 418
<211> 379
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 418

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
35 40 45

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val
130 135 140

Gln Pro Gly Asn Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
145 150 155 160

Phe Ser Ser Phe Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly
165 170 175

Leu Glu Trp Val Ser Ser Ile Ser Gly Ser Gly Ser Asp Thr Leu Tyr
180 185 190

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
195 200 205

Thr Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala
210 215 220

Val Tyr Tyr Cys Thr Ile Gly Gly Ser Leu Ser Arg Ser Ser Gln Gly
225 230 235 240

Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser
245 250 255

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
260 265 270

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Arg Ser Leu Ser Asn Tyr
275 280 285

Tyr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Glu Leu Leu
290 295 300

Gly Asn Ile Ser Trp Arg Gly Tyr Asn Ile Tyr Tyr Lys Asp Ser Val
305 310 315 320

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Ile Tyr
325 330 335

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
340 345 350

Ala Ala Ser Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
355 360 365

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
370 375

<210> 419
<211> 260
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 419

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
145 150 155 160

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp
165 170 175

Tyr Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
180 185 190

Val Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser
195 200 205

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu
210 215 220

Tyr Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr
225 230 235 240

Cys Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val
245 250 255

Thr Val Ser Cys
260

<210> 420
<211> 264
<212> PRT
<213> Artificial

<220>
<223> constructo de nanocuerpo

<400> 420

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
145 150 155 160

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp
165 170 175

Tyr Trp Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
180 185 190

Val Ser Glu Ile Asn Thr Asn Gly Leu Ile Thr Lys Tyr Pro Asp Ser
195 200 205

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu
210 215 220

Tyr Leu Gln Met Asn Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr
225 230 235 240

Cys Ala Arg Ser Pro Ser Gly Phe Asn Arg Gly Gln Gly Thr Leu Val
245 250 255

Thr Val Ser Ser Gly Gly Gly Cys
260

<210> 421
<211> 20
<212> DNA
<213> Artificial

<220>
<223> cebador de oligonucleótido

<400> 421
ggataacaat ttcacacagg 20

<210> 422
<211> 55
<212> DNA
<213> Artificial

<220>
<223> cebador de oligonucleótido

<400> 422
tcagtaacct ggatccgcca ccgctgcctc caccgcctga ggagacggtg accag 55

<210> 423
<211> 39
<212> DNA
<213> Artificial

<220>
<223> cebador de oligonucleótido

<400> 423
aggttactga ggatccgagg tgcagctggt ggagtctgg 39

<210> 424
<211> 46
<212> DNA
<213> Artificial

<220>
<223> cebador de oligonucleótido

<400> 424
tcagtaacct tccggaaccg ccaccgcctg aggagacggt gacaag 46

<210> 425
<211> 48
<212> DNA
<213> Artificial

<220>
<223> cebador de oligonucleótido

<400> 425
aggttactga tccggaggcg gtagcgaggt gcagctggtg gagtctgg 48

<210> 426
<211> 19
<212> DNA
<213> Artificial

<220>
<223> cebador de oligonucleótido

<400> 426
cacgacgttg taaaacgac 19

<210> 427
<211> 43
<212> DNA
<213> Artificial

<220>
<223> cebador de oligonucleótido

<400> 427
atggtggtgt gcggccgcct attatgagga gacggtgacc agg 43

<210> 428
<211> 45
<212> DNA
<213> Artificial

<220>
<223> cebador de oligonucleótido

<400> 428
aggggtatct ctcgagaaaa gagaggtgca gctggtggag tctgg 45

1406

<210> 429
<211> 56
<212> DNA
<213> Artificial

<220>
<223> cebador de oligonucleótido

<400> 429
gctaaagaag aaggggtatc tctcgagaaa agagaggtgc agctggtgga gtctgg 56

<210> 430
<211> 88
<212> DNA
<213> Artificial

<220>
<223> cebador de oligonucleótido

<400> 430
tcagtaacct ggatcccccg ccaccgctgc ctccaccgcc gctacccccg ccaccgctgc 60

ctccaccgcc tgaggagacg gtgacaag 88

<210> 431
<211> 69
<212> DNA
<213> Artificial

<220>
<223> cebador de oligonucleótido

<400> 431
aggttactga ggatccggcg gtggaggcag cggtggcggg ggtagcgagg tgcagctggt 60

ggagtctgg 69

<210> 432
<211> 41
<212> DNA
<213> Artificial

<220>
<223> cebador de oligonucleótido

<400> 432
atggtggtgt gaattcttat tagcaggaga cggtgacaag g 41

<210> 433
<211> 53

<212> DNA
<213> Artificial

<220>
<223> cebador de oligonucleótido

<400> 433
atggtggtgt gaattcttat tagcaacctc cacctgagga gacggtgaca agg 53

<210> 434
<211> 21
<212> DNA
<213> Artificial

<220>
<223> cebador de oligonucleótido

<400> 434
gactggttcc aattgacaag c 21

<210> 435
<211> 21
<212> DNA
<213> Artificial

<220>
<223> cebador de oligonucleótido

<400> 435
gcaaatggca ttctgacatc c 21

<210> 436
<211> 13
<212> PRT
<213> Artificial

<220>
<223> Elemento CDR3

<400> 436

Ile Leu Pro Leu Ser Asp Asp Pro Gly Trp Asn Thr Tyr
1 5 10

<210> 437
<211> 4
<212> PRT
<213> Artificial

<220>

148
1408

<223> elemento de nanocuerpo

<400> 437

Lys Glu Arg Glu
1

<210> 438
<211> 4
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 438

Lys Gln Arg Glu
1

<210> 439
<211> 4
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 439

Gly Leu Glu Trp
1

<210> 440
<211> 5
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 440

Lys Glu Arg Glu Leu
1 5

<210> 441
<211> 5
<212> PRT
<213> Artificial

10
1409

<220>
<223> elemento de nanocuerpo

<400> 441

Lys Glu Arg Glu Phe
1 5

<210> 442
<211> 5
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 442

Lys Gln Arg Glu Leu
1 5

<210> 443
<211> 5
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 443

Lys Gln Arg Glu Phe
1 5

<210> 444
<211> 5
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 444

Lys Glu Arg Glu Gly
1 5

<210> 445
<211> 4
<212> PRT

<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 445

Thr Glu Arg Glu
1

<210> 446
<211> 5
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 446

Thr Glu Arg Glu Leu
1 5

<210> 447
<211> 4
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 447

Lys Glu Cys Glu
1

<210> 448
<211> 5
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 448

Lys Glu Cys Glu Leu
1 5

<210> 449

<211> 5
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 449

Lys Glu Cys Glu Arg
1 5

<210> 450
<211> 4
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 450

Arg Glu Arg Glu
1

<210> 451
<211> 5
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 451

Arg Glu Arg Glu Gly
1 5

<210> 452
<211> 4
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 452

Gln Glu Arg Glu
1

<210> 453

1412

<211> 5
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 453

Gln Glu Arg Glu Gly
1 5

<210> 454
<211> 4
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 454

Lys Gly Arg Glu
1

<210> 455
<211> 5
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 455

Lys Gly Arg Glu Gly
1 5

<210> 456
<211> 4
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 456

Lys Asp Arg Glu
1

<210> 457
<211> 5
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 457

Lys Asp Arg Glu Val
1 5

<210> 458
<211> 5
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 458

Asp Glu Cys Lys Leu
1 5

<210> 459
<211> 5
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 459

Asn Val Cys Glu Leu
1 5

<210> 460
<211> 4
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 460

Gly Val Glu Trp
1

<210> 461
<211> 4
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 461

Glu Pro Glu Trp
1

<210> 462
<211> 4
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 462

Gly Leu Glu Arg
1

<210> 463
<211> 4
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 463

Asp Gln Glu Trp
1

<210> 464
<211> 4
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 464

Asp Leu Glu Trp
1

<210> 465
<211> 4

<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 465

Gly Ile Glu Trp
1

<210> 466
<211> 4
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 466

Glu Leu Glu Trp
1

<210> 467
<211> 4
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 467

Gly Pro Glu Trp
1

<210> 468
<211> 4
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 468

Glu Trp Leu Pro
1

<210> 469
<211> 4
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 469

Gly Pro Glu Arg
1

<210> 470
<211> 4
<212> PRT
<213> Artificial

<220>
<223> elemento de nanocuerpo

<400> 470

Gly Leu Glu Arg
1

<210> 471
<211> 4
<212> PRT
<213> Artificial

<220>
<223> espaciador

<400> 471

Gly Gly Gly Cys
1

<210> 472
<211> 109
<212> PRT
<213> Artificial

<220>
<223> constructo de inmunoglobulina

<400> 472

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Ser Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105

<210> 473
<211> 109
<212> PRT
<213> Artificial

<220>
<223> constructo de inmunoglobulina

<400> 473

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Trp Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Val Trp Val
35 40 45

Ser Arg Ile Asn Ser Asp Gly Ser Ser Thr Ser Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

1418

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105

<210> 474
<211> 109
<212> PRT
<213> Artificial

<220>
<223> constructo de inmunoglobulina

<400> 474

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105

<210> 475
<211> 111
<212> PRT
<213> Artificial

<220>
<223> constructo de inmunoglobulina

1419

<400> 475

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp His
20 25 30

Tyr Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Arg Thr Arg Asn Lys Ala Asn Ser Tyr Thr Thr Glu Tyr Ala Ala
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Ala Arg Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105 110

<210> 476
<211> 17
<212> PRT
<213> Artificial

<220>
<223> enlazador

<400> 476

Ala Ala Ala Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Gly Ala
1 5 10 15

Ala

1420

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/004678

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/18 C07K16/24 A61P29/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/041862 A2 (ABLYNX N V [BE]; SILENCE KAREN [BE]; LAUWEREYS MARC [BE]; DE HAARD HAN) 21 May 2004 (2004-05-21) page 66; examples 2-4	1-11

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 December 2006	04/01/2007

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Wagner, René

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/004678

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	COPPIETERS KEN ET AL: "Formatted anti-TNF-alpha nanobodies show superior efficacy in a collagen-induced arthritis model in mice." ARTHRITIS & RHEUMATISM, vol. 52, no. 9, Suppl. S, September 2005 (2005-09), pages S362-S363, XP009075962 & 69TH ANNUAL SCIENTIFIC MEETING OF THE AMERICAN-COLLEGE-OF-RHEUMATOLOG Y/40TH ANNUAL SCIENTIFIC MEETIN; SAN DIEGO, CA, USA; NOVEMBER 12 -17, 2005 ISSN: 0004-3591 abstract	1-111
A	RIECHMANN L ET AL: "Single domain antibodies: comparison of camel VH and camelised human VH domains" JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL, vol. 231, no. 1-2, 10 December 1999 (1999-12-10), pages 25-38, XP004187632 ISSN: 0022-1759 the whole document	1-111
T	COPPIETERS KEN ET AL: "Formatted anti-tumor necrosis factor alpha VHH proteins derived from camelids show superior potency and targeting to inflamed joints in a murine model of collagen-induced arthritis" ARTHRITIS & RHEUMATISM, vol. 54, no. 6, June 2006 (2006-06), pages 1856-1866, XP002410952 ISSN: 0004-3591 the whole document	1-111
P,A	CONRATH K ET AL: "Antigen Binding and Solubility Effects upon the Veneering of a Camel VHH in Framework-2 to Mimic a VH" JOURNAL OF MOLECULAR BIOLOGY, LONDON, GB, vol. 350, no. 1, 1 July 2005 (2005-07-01), pages 112-125, XP004918534 ISSN: 0022-2836 the whole document	1-111
A	WO 03/035694 A2 (VLAAMS INTERUNIV INST BIOTECH [BE]; MUYLDERMANS SERGE [BE]) 1 May 2003 (2003-05-01) example 6	1-111

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2006/004678

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004041862 A2	21-05-2004	WO 2004041865 A2	21-05-2004
		WO 2004041863 A2	21-05-2004
		CA 2505316 A1	21-05-2004
		CA 2505325 A1	21-05-2004
		CA 2505326 A1	21-05-2004
		JP 2006519763 T	31-08-2006
		JP 2006524036 T	26-10-2006
		JP 2006520584 T	14-09-2006
		MX PA05004955 A	17-11-2005
WO 03035694 A2	01-05-2003	CA 2464372 A1	01-05-2003
		JP 2005520494 T	14-07-2005

1423

Industria y Comercio
SUPERINTENDENCIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES
EXTRACTO PARA PUBLICACIÓN
SOLICITUD DE PATENTE DE INVENCIÓN PCT

(21) N° de solicitud: 22) Fecha de solicitud: (30) Prioridad (31) ***Prioridad*** (32) ***Fecha*** (33) ***País*** **60/682,332** **18/05/2005** **US**	(51) Int Cl: **C07K 016/018** (71)Solicitante(s) **ABLYNX N.V.** (72) Inventor(es) **ELS BEIRNAERT** (74) Apoderado: **EMILIO FERRERO**
(85) Fecha limite inicio fase nacional: 18/12/0007 (86) Datos relativos a la presentación de la solicitud PCT Fecha de presentación de la solicitud: 17/05/2006 No. de solicitud PCT/EP2006/004678	(87) Publicación internacional Fecha: 23/11/2006 N° publicación: ***WO 2006/122786 A3***

(54) Titulo: **NANOBODIES™ (NANOCUERPOS) MEJORADOS CONTRA EL FACTOR ALFA DE NECROSIS DEL TUMOR**

Reivindicación(es):

1. Nanocuerpo contra TNF-alfa, el cual consiste de 4 regiones de estructura (FR1 hasta FR4 respectivamente) y 3 regiones que determinan la complementariedad (CDR1 hasta CDR3 respectivamente), caracterizado porque:

d) CDR1 comprende:

- la secuencia de aminoácido DYWMY; o
- una secuencia de aminoácido que tiene, al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido DYWMY; o
- secuencias de aminoácido que tienen solamente 1 diferencia de aminoácido con la secuencia de aminoácido DYWMY;

y

e) CDR2 comprende:

- la secuencia de aminoácido EINTNGLITKYPDSVKG; o
- una secuencia de aminoácido que tiene, al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido EINTNGLITKYPDSVKG; o
- secuencias de aminoácidos que tienen 2 ó solamente 1 diferencia(s) de aminoácido(s) con la secuencia de aminoácido EINTNGLITKYPDSVKG; y

f) CDR3 comprende:

- la secuencia de aminoácido SPSGFN; o
- una secuencia de aminoácido que tiene, al menos 80%, preferiblemente al menos 90%, más preferiblemente al menos 95%, aún más preferiblemente al menos 99% de identidad de secuencia con la secuencia de aminoácido SPSGFN; o
- secuencias de aminoácido que tienen solamente 1 diferencia de aminoácido con la secuencia de aminoácido SPSGFN.

2. Nanocuerpo de conformidad con la reivindicación 1, caracterizado porque CDR1 comprende la secuencia de aminoácido DYWMY.

3. Nanocuerpo de conformidad con la reivindicación 1, caracterizado porque CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG.

4. Nanocuerpo de conformidad con la reivindicación 1, caracterizado porque CDR3 comprende la secuencia de aminoácido SPSGFN.

5. Nanocuerpo de conformidad con la reivindicación 1, caracterizado porque:

- CDR1 comprende la secuencia de aminoácido DYWMY; y
- CDR3 comprende la secuencia de aminoácido SPSGFN; o
- CDR1 comprende la secuencia de aminoácido DYWMY; y CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG; o
- CDR2 comprende la secuencia de aminoácido EINTNGLITKYPDSVKG; y CDR3 comprende la secuencia de aminoácido SPSGFN

1924

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 07-121403- -00000-0000

Fecha: 2007-11-16 17:44:01 Dep. 2020 NUECREACIONI
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 1424

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 07 - 91,012
FECHA : NOVIEMBRE 7 DE 2007

***** C O N S I G N A C I O N *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
CAVELIER ABOGADOS	CONSIGNACION	BANCO POPULAR	050-00110-6	48825373	07/11/2007	48,438,000.00

***** C O N C E P T O *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE IN	482,000.00
		TOTAL : $	482,000.00

SON: CUATROCIENTOS OCHENTA Y DOS MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No._____

COLO. 16204-841-001-1

1425

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 07-121403- -00000-0000

Fecha: 2007-11-16 17:44:01 Dep: 2020 NUECREACIONE
Tra: 11 PATENTEIYII Eve: 378 FASENACIONALI
Act: 411 PRESENTACION Folios: 1424

Industria y Comercio
SUPERINTENDENCIA

REQUISITOS MINIMOS PARA ADMISION A TRAMITE PCT

PATENTE DE INVENCION [✓] MODELO DE UTILIDAD [ ]

- Indicación de que se solicita la concesión de una patente [✓]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [✓]
- Descripción de la invención [✓]
- Dibujos de ser estos pertinentes [ ]
- ◆ Comprobante de pago de las tasas establecidas [✓]

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_____

Carrera 13 No. 27-00 Piso 5, 7 y 10
Conmutador: 3 82 08 40
Fax: 350 52 20
E-mail: info@sic.gov.co
www.sic.gov.co
Bogotá, D.C., Colombia